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Relation of the Rate of Penetration and Metabolism to the Toxicity of Sevin to Three Insect Species[1]

M. E. Eldefrawi and W. M. Hoskins, *University of California, Berkeley*

Abstract

Sevin® (1-naphthyl N-methylcarbamate) labelled with C^{14} has been used on three insect species by topical application in acetone. Sevin is absorbed rapidly into house flies (*Musca domestica* L.), 75% of a dose penetrating within 4 hours. It is rapidly metabolized and excreted, so final toxicity is low as shown by the LD_{50}=2.0 μg./♀. Resistant house flies differ from susceptible ones only in greater metabolism and consequent lower mortality. If metabolism is prevented by addition of a synergist such as sesamex, the toxicity of Sevin is increased up to fifty-fold, and much of the absorbed Sevin remains in the body unchanged. The critical step is the hydrolysis of Sevin to 1-naphthol and methyl amine, which is controlled by a carbamate esterase enzyme. When this is inhibited, *e.g.*, by sesamex, the toxicity is high.

Sevin penetrates more slowly into the large milkweed bug (*Oncopeltus fasciatus* (Dall.)), *e.g.*, 40% of an applied dose enters in 8 hours. It is metabolized and excreted very slowly so that LD_{50} is low, *i.e.*, 0.5 μg./insect. The German cockroach (*Blattella germanica* (L.)) absorbs Sevin slowly and metabolizes it rapidly. This results in low toxicity, LD_{50}=20 μg./♂. The metabolic products are different in the three species and are formed by different reactions.

Considerable evidence is available to show that resistance to the organic phosphate insecticides is due in part at least to metabolism of the compounds or of their activated oxygen analogues to nontoxic derivatives. Van Asperen & Oppenoorth (1960) found a higher detoxication rate in organophosphate-resistant than in normally susceptible house flies. Metcalf (1959) pointed out that detoxication of para-oxon could be catalyzed by a para-oxon-insensitive aromatic esterase. Such an enzyme has been found by Metcalf *et al.* (1956) to be abundant in honey bee abdomens and in cockroach guts, and it is able to hydrolyze para-oxon about half as readily as phenyl acetate. Further support for the role of detoxication in organophosphate-resistance was obtained by March (1959) with a highly malathion-resistant strain of flies which he found to metabolize malaoxon twice as fast as a normal strain did. O'Brien (1957) found that the degradation of malathion is hydrolytic and is caused either by a carboxy-esterase removing one or both of the ethyl esters of the malic acid moiety or by "phosphatase" splitting of dimethyl thiophosphate or phosphate. Krueger & O'Brien (1959) found the latter process to predominate in the normal house fly.

There is much less experimental data on the carbamate insecticides. It was suggested that the inertness of Pyrolan after ingestion by *Periplaneta americana*, *Melolantha melolantha*, and certain lepidopterous larvae might be due to its destruction in the gut (Wiesmann & Kocher 1951). Pyrolan was injected into the cockroach heart and its distribution and stability determined by house fly bioassay; no toxicant was left in the blood after 1 hour, but after 2 to 4 hours, toxic products could still be detected in the gut, fat body, muscles, and thoracic ganglia. From a

Table 1.—Toxicity of Sevin and of Sevin with sesamex applied topically to three species of insects.

Species	Sevin (μg./insect)	Sesamex (μg./insect)	Mortality[a] (%)
House flies[b]			
SCR	0.5	—	10
	1.0	—	20
	2.0	—	52
	5.0	—	70
	0.5	0.05	64
	0.5	0.1	76
	0.5	0.2	96
PR	30.0	—	10
	2.0	0.5	48
	2.0	1.0	90
Large milkweed bug	0.125	—	10
	0.25	—	20
	0.5	—	40
	1.0	—	100
	0.125	1.0	30
German cockroach	7.5	—	10
	15.0	—	40
	0.1	5.0	10
	0.5	5.0	90

[a] Mortality was determined after 24 hours with the house flies and after 48 hours with the other insects.

[b] SCR, normally susceptible strain; PR, tenfold resistant parathion strain.

[1] Contribution from the Laboratory of Insect Physiology and Toxicology, Department of Entomology and Parasitology, University of California, Berkeley, California.

This report is based upon experimental work financed in part under Research Grant E-1081 from the U. S. Public Health Service. Accepted for publication October 25, 1960.

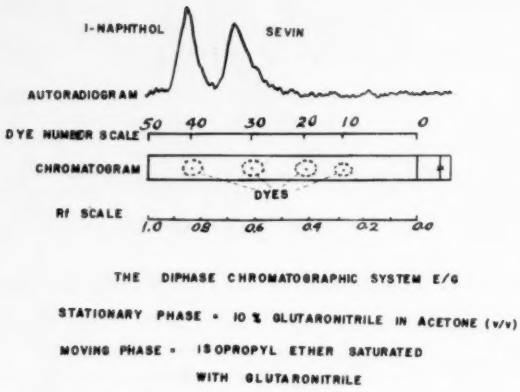

FIG. 1.—Standard reference chromatograms of C^{14}-Sevin and 1-Naphthol-C^{14}

study on dimethyl carbamates of heterocyclic alcohols, it was concluded that the contrast of *in vitro* anticholinesterase activity with *in vivo* toxicity might be partially due to *in vivo* metabolism. Eldefrawi *et al.* (1960) arrived at the same conclusion working with Sevin and three dimethyl carbamates of heterocyclic alcohols.

INSECTS AND EXPERIMENTAL METHODS.—This report concerns the toxicity of the carbamate insecticide Sevin® (1-naphthyl N-methylcarbamate) to normal and parathion-resistant house flies (*Musca domestica L.*), to the German cockroach (*Blattella germanica* (L.)), and to the large milkweed bug (*Oncopeltus fasciatus* (Dall.)); the synergistic effect upon these of sesamex, acetaldehyde 2-(2-ethoxyethoxy) ethyl 3,4-methylenedioxyphenyl acetal; and the metabolism of Sevin in the three species. The flies were a normally susceptible strain (SCR) reared in the laboratory for several years—LD_{50} for DDT, 0.2 μg./ ♀, LD_{50} for Sevin, 2 μg./ ♀; and a tenfold resistant parathion strain (PR) secured from the California Citrus Experiment Station at Riverside—LD_{50} for Sevin, approximately 50μg./ ♀. The German cockroaches were a normal laboratory strain—LD_{50} for Sevin, 20 μg./ ♂; and the milkweed bugs were a normal strain obtained from the Stauffer Chemical Company—LD_{50} for Sevin, 0.5 μg./last-instar nymph. All dosages were applied topically in acetone under light CO_2 anesthesia. The treated insects were provided with food and water, and mortality was determined after 24 hours with the flies and 48 hours with the other insects.

RESULTS.—When Sevin is applied with sesamex, there is a synergism with all strains, but much more marked with the resistant flies. Table 1 shows a few representative data. The LD_{50} for susceptible flies is reduced from 2 μg. for Sevin alone to slightly less than 0.5 μg. with 0.05 μg. sesamex present. With the resistant strain, the effect is more marked, *e.g.*, LD_{50} changes from approximately 50 μg. to 2 μg. with 0.5 μg. sesamex, and to 1 μg. with 1.0 μg. sesamex present.

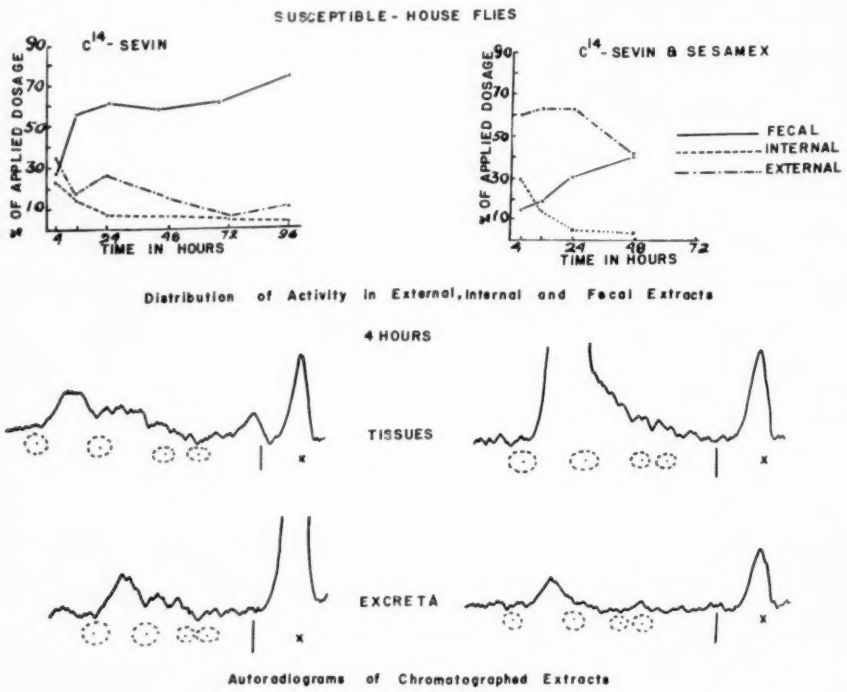

FIG. 2.—Absorption and metabolism curves and chromatograms of extracts from susceptible house flies treated with C^{14}-Sevin.

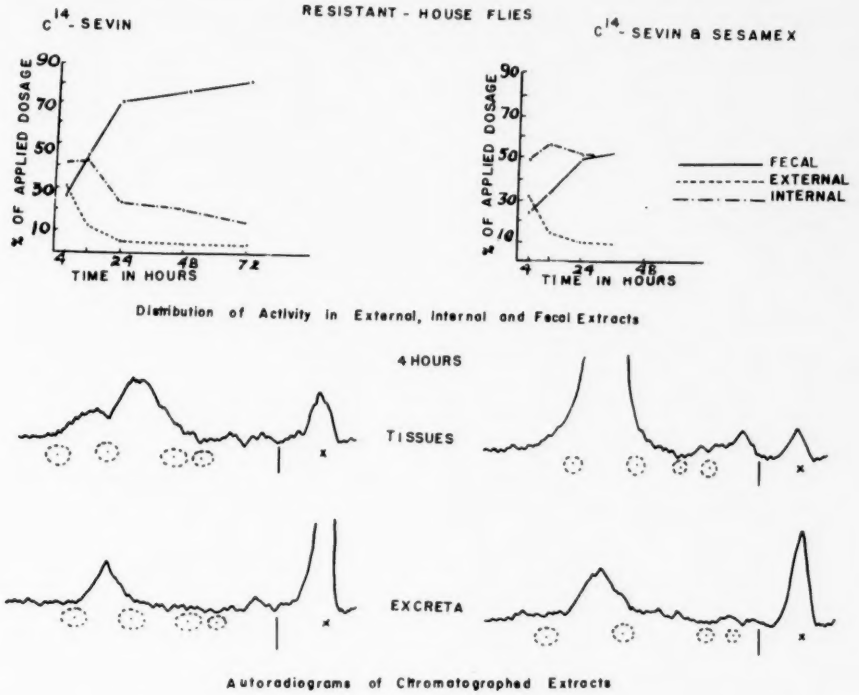

FIG. 3.—Absorption and metabolism curves and chromatograms of extracts from resistant house flies treated with C^{14}-Sevin.

Synergism is much lower in the case of the milkweed bug nymph, *e.g.*, the 10% kill from 0.125 μg. Sevin alone increases only to 30% with 1.0 μg. sesamex present. In the cockroach, the synergistic effect is higher, LD_{50} dropping from 20 μg. to 0.4 μg. with 5 μg. sesamex present. It will be obvious that a strong synergism exists with Sevin and sesamex, but unlike many other combinations, this one has the disadvantage that the toxicant is cheap while the added material is relatively expensive. Hence, its practical use is limited.

Since the parathion-resistant strain of flies was also resistant to Sevin, it was of special interest to compare the metabolic fate of Sevin in the two strains. For this purpose, C^{14}-Sevin was synthesized by reacting 1-naphthol-C^{14} with methyl isocyanate. At intervals after application, the insects were rinsed in acetone to remove unpenetrated Sevin, the homogenized tissues were extracted with acetone, then with ether-alcohol, and the excreta in the holding cage was extracted with hot methanol and acetone. All extracts were concentrated at low temperature and dissolved in acetone for paper chromatography.

Chromatography was done on 1" paper strips of Whatman No. 4 filter paper with glutaronitrile as stationary phase and isopropyl ether (saturated with glutaronitrile) as ascending mobile phase. Location of radioactive peaks was expressed in terms of location with respect to a system of dyes giving four spots which are well placed in this solvent system and furnish much more reliable reference points than the usual Rf numbers since these are seriously displaced by lipoid materials present in most tissue extracts. The system is exemplified in figure 1 which shows the peaks of radioactivity obtained by applying C^{14}-Sevin and 1-naphthol-C^{14} and scanning the developed chromatogram with a gas flow Geiger-Müller tube. In this chromatographic system, the lower the polarity of a solute, the farther it will move.

Figure 2 shows two kinds of data: a) the changes in total amount of external, internal and excreted activity at intervals after application of C^{14}-Sevin to the susceptible house flies, and b) chromatograms of tissue and excreta extracts 4 hours after application (on the left hand side) and the same when sesamex was added (on the right hand side). Evidently Sevin penetrated rapidly either alone or with sesamex, but there is a very large effect of the synergist in increasing the amount of internal Sevin and in decreasing the activity in the excreta, *i.e.*, sesamex interferes with the process(es) by which absorbed Sevin is metabolized and excreted This is also shown by the chromatograms. Sevin alone is rapidly changed to a highly polar substance staying at the point of application to the paper strip. This substance occurs in both the tissues and the excreta. With sesamex present there is a much greater amount of unchanged Sevin in the body and much less polar metabolite in the excreta.

With the resistant house fly (fig. 3) the total activity of external, internal and excreta extracts is very nearly the same as in the susceptible strain, both for Sevin alone and

plus sesamex. Hence the differences in susceptibility and the synergism cannot be due to changes in absorption. Also the chromatograms are very similar to those for the susceptible flies except that more of the polar material is excreted and less Sevin remains in the body. There is thus a *quantitative* difference in rates of metabolism favoring survival of the resistant strain. Sesamex decreases the excretion of metabolites and increases the retention of unchanged Sevin in the bodies of resistant flies in exactly the same manner as with the susceptible ones. At later periods, the same relations hold for both strains with and without sesamex present. Accordingly, the data are not presented here.

Figure 4 shows that the milkweed bug nymph absorbs Sevin fairly rapidly but excretes radioactive products very inefficiently. The chromatograms show only unchanged Sevin and the very polar metabolite to be present in measureable amounts in either tissues or excreta at 4 and 15 hours. It is obvious that at the latter period the continued absorption has accumulated a very large amount of unchanged Sevin in the bug's body, whereas excretion has not changed much. The same situation prevails when sesamex is added. The high susceptibility of the milkweed bug to Sevin clearly correlates with low metabolism of the chemical.

The German cockroach (fig. 5) presents a more complicated metabolic picture with a total of six products shown repeatedly on chromatograms of tissue and excreta extracts. The rather slow absorption and rapid rate of excretion account for the failure to accumulate large amounts of radioactive material in the tissues. The most significant features are the steady decrease in unchanged Sevin in the tissues and the accompanying increase in metabolic products in the excreta. 1-Naphthol is evident in the tissues at 72 hours, and especially in the excreta at 24 and 72 hours. This substance was not found in appreciable amounts with the other insects.

DISCUSSION.—It is clear that the metabolic pattern

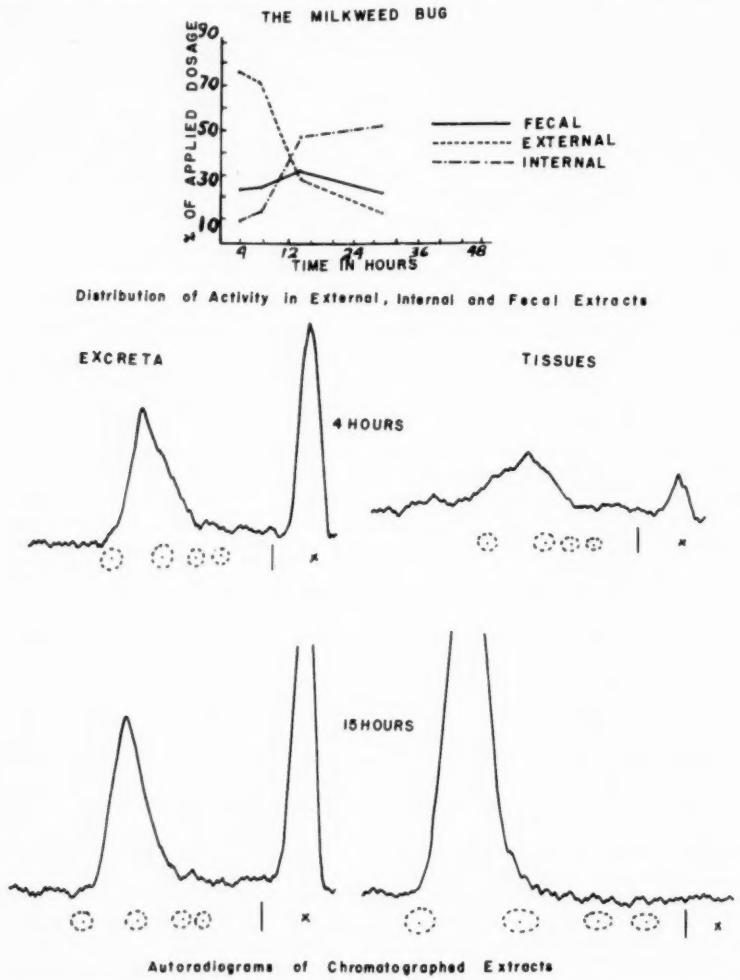

FIG. 4.—Absorption and metabolism curves and chromatograms of extracts from large milkweed bugs treated with C^{14} Sevin.

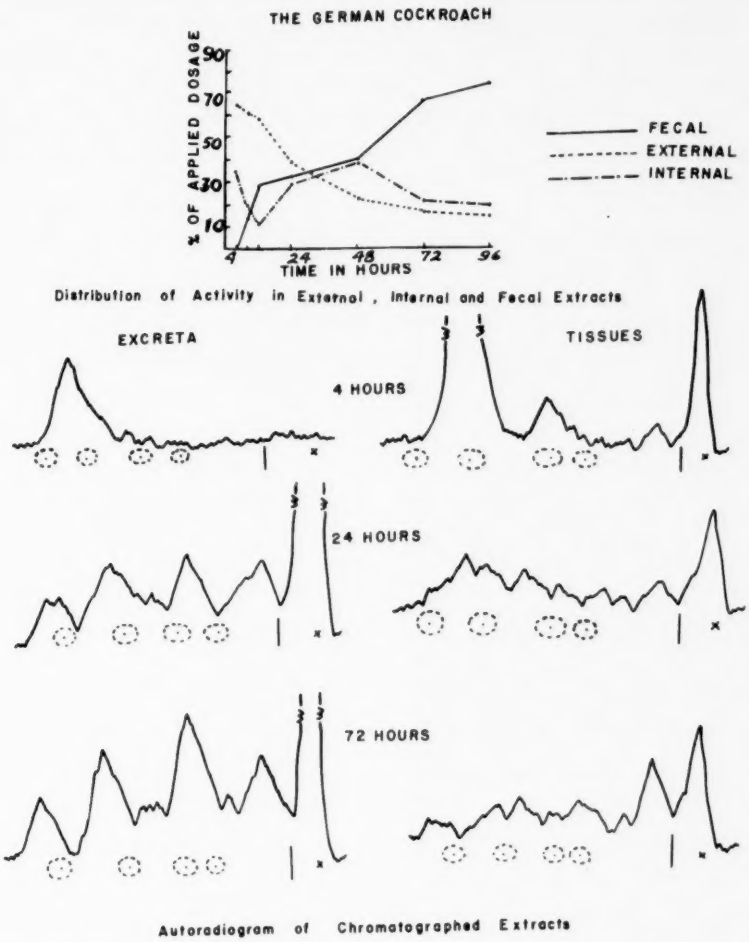

FIG. 5.—Absorption and metabolism curves and chromatograms of extracts of German cockroaches treated with C^{14}-Sevin.

differs greatly in the three insects, being simplest in the milkweed bug which forms only a very polar product, more complicated in the house fly in which at least three metabolites can be distinguished, and most elaborate in the German cockroach with six metabolites. However, one of these three or six probably is the key product whose rate of formation controls the overall metabolism. Comparison with the changes undergone by 1-naphthol in these insects shows it to form the same series of more polar products. Hence a working hypothesis is that the first step in the metabolism of Sevin is hydrolysis of the ester bond. Further identification of the steps and the products is being studied at the present time.

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Toxicity to Face Fly and House Fly Larvae of Feces from Insecticide-Fed Cattle[1]

DARRELL W. ANTHONY,[2] NORMAN W. HOOVEN,[3] and OTELIA BODENSTEIN,[2,4] *Agric. Res. Serv., U.S.D.A.*

ABSTRACT

Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate), and ronnel were administered to Holstein heifers in the grain ration for 5 consecutive days to determine whether the treated animals would produce droppings that were toxic to the larvae of face flies (*Musca autumnalis* DeGeer) and house flies (*Musca domestica* L.). Tests in which the feces from the treated animals were infested with newly hatched larvae showed that Co-Ral and Bayer 22408 completely inhibited the development of face fly larvae at dosages of 1.0 and 0.5 milligrams per kilogram of animal weight. House fly larvae failed to survive at the 1.0 mg./kg. dosages of both chemicals. The 0.5 mg./kg. dosages were highly effective, but did not completely stop house fly development. Feces from animals fed ronnel at 5.0 mg./kg. gave complete kill of face fly and house fly larvae. Ronnel at 2.5 mg./kg. was effective against the larvae of face flies, but not house flies.

Since the discovery of the face fly (*Musca autumnalis* DeGeer) in Nova Scotia, Canada, in 1952, it has spread and become troublesome in the Middle Atlantic and some Midwestern States. Face flies attack the moist membranes around the eyes, nose, and mouth of cattle and cause severe annoyance and, consequently, improper meat and milk gains. Pyrethrins and related compounds have not provided effective control. Bruce *et al.* (1960) reported that residues from the treatment of farm premises and animals failed to effect significant face fly control. However, baits containing dimethoate and DDVP applied to the foreheads of cattle did provide effective control.

Face flies breed principally in fresh cattle droppings. Thus it seems probable that low-level feeding of insecticides to cattle might provide effective control through feces contamination. Early work by Knipling (1938) and Bruce (1939) showed that horn flies failed to develop in the droppings of cattle fed or given oral drenchings of phenothiazine. Eddy *et al.* (1954) reported the effect on fly larvae of manure from cattle fed several chlorinated hydrocarbon insecticides. Recent investigations by Eddy & Roth of the Corvallis, Ore., laboratory of the Entomology Research Division (1961) showed that cattle fed some of the newer organophosphorus insecticides produced feces that were toxic to larvae of the house fly (*Musca domestica* L.), horn fly (*Siphona irritans* (L.)), and stable fly (*Stomoxys calcitrans* (L.)). Because of this promising work, experiments were initiated at Beltsville, Md., to determine whether feeding certain insecticides to cattle would produce droppings that were toxic to face fly larvae. Comparative tests were also conducted with house fly larvae.

METHODS AND MATERIALS.—The experimental animals were 16-to-18-month-old Holstein heifers weighing between 820 and 950 pounds. Six animals were selected for testing and another as a control to receive a similar untreated ration. The animals were weighed on 2 consecutive days to obtain an average weight for insecticide-dosage calculation. Four days prior to the test period, the animals were placed on the ration they were to receive during the experiment. The ration consisted of 4 pounds of a grain mixture containing cracked corn, crimped oats, wheat bran, ground barley, dried molasses, mineralized salt, and steamed bone meal. This ration contained 70.5% total digestible nutrients, or 72.5 therms of estimated net energy per 100 pounds of feed. In addition each animal received 20 pounds of alfalfa hay daily. Records on the grain and hay consumed were taken each day. The animals were stanchioned throughout the experimental period except for about 2 hours each day when they were turned out for water.

Two experiments were conducted in which Co-Ral (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate), and ronnel were administered in the grain ration for 5 consecutive days. The dosages, based on milligrams per kilogram of animal weight, are given in table 1. Two animals were used for each dosage of Bayer 22408 and ronnel; however, only one animal was used for each dosage with Co-Ral. In the second experiment each animal was fed a different insecticide than it had received in the first. The insecticides were dissolved in 50 ml. of acetone and then applied to the ration.

Daily fecal collections were made during the pretreatment, treatment, and posttreatment periods. Samples of about 500 grams were taken manually from the rectum of each animal approximately 18 hours after the treated ration was given. The samples were placed in pint-sized ice-cream cartons, labeled, and returned to the laboratory for immediate testing.

The test containers were wide-mouthed pint-sized mason jars fitted with muslin tops. About 10 grams of wood shavings was placed in the bottom of each jar over which 100 grams of feces was introduced. The feces were then infested with newly hatched face fly or house fly larvae. Two or three days later, additional wood shavings were placed on top of the manure to serve as a pupation medium.

Face fly larvae were obtained by egging field-collected adults. Collections were made by netting from newborn calves and old cows that were not disturbed by the net. A few flies were also collected from fences, trees, buildings,

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[2] Entomology Research Division.
[3] Animal Husbandry Research Division.

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Table 1.—Flies emerging from the feces of cattle receiving three organophosphorus insecticides in the ration.

Insecticide and Dosage (mg./kg.)	Treatment				Posttreatment					
	Per Cent Emerged on Days Shown				Per Cent Emerged on Days Shown					
	1	2	3	4	1	2	3	4	5	8
Experiment 1										
Face flies										
Bayer 22408, 1.0	0	0	0	0	0	0	0	—	75	92
1.0	0	0	0	0	0	0	100	—	90	100
Ronnel, 5.0	98	0	0	0	10	0	40	—	95	88
5.0	0	0	0	0	0	0	60	—	75	98
Co-Ral, 2.5	0	0	0	0	0	0	0	—	90	78
1.0	0	0	0	0	0	0	0	—	80	85
Untreated	94	64	90	100	85	95	100	—	80	95
House flies										
Bayer 22408, 1.0	0	0	0	0	—	0	16	—	84	—
1.0	0	0	0	0	—	0	46	—	79	—
Ronnel, 5.0	—[a]	24	27	22	—	25	51	—	74	—
5.0	0	0	0	0	—	9	53	—	92	—
Co-Ral, 2.5	0	0	0	0	—	0	0	—	87	—
1.0	0	0	0	0	—	0	20	—	94	—
Untreated	100	99	91	74	—	100	85	—	100	—
Experiment 2										
Face flies										
Bayer 22408, 0.5	0	0	0	0	0	—	100	90	—	—
.5	0	0	0	0	0	—	100	95	—	—
Ronnel, 2.5	0	0	0	0	0	—	80	75	—	—
2.5	0	0	0	0	0	—	100	95	—	—
Co-Ral, 1.0	0	0	0	0	0	—	0	80	—	—
0.5	0	0	0	0	0	—	40	85	—	—
Untreated	80	85	93	95	90	—	70	90	—	—
House flies										
Bayer 22408, 0.5	18	0	1	5	18	59	98	95	—	—
.5	7	0	1	0	3	45	100	93	—	—
Ronnel, 2.5	28	11	38	48	17	47	100	100	—	—
2.5	100	7	45	11	14	100	100	100	—	—
Co-Ral, 1.0	8	0	0	0	0	1	82	100	—	—
0.5	13	0	0	1	1	25	83	84	—	—
Untreated	85	61	74	100	95	89	96	94	—	—

[a] Discarded.

and other inanimate objects. The adults were returned to the laboratory and placed in a cage measuring 2×2×3 feet. The flies were fed a milk and sucrose solution and malt syrup was also offered on small cards distributed on the bottom of the cage. A small dish of fresh cow manure was placed near the top of the cage to attract females ready to oviposit. The best egging results were attained when the cage was taken outdoors into bright, warm sunshine. New flies from the field were added to the cage nearly every day during the test period. The larvae were separated by placing 3 to 5 grams of the manure in the bottom of a white enamel pan, then slowly separating the material with a stream of distilled water from a polyethylene squeeze bottle. As the larvae were washed out they were picked up with an eyedropper and transferred to the test jars. Two jars each containing 10 or 20 larvae were used in all but one of the tests. One jar containing 10 larvae was used in the test conducted on the first day after treatment in the second experiment.

House-fly larvae were obtained by egging 4- or 5-day-old adults of a nonresistant colony. A wad of cotton saturated with a milk and sucrose solution was placed in the cage on a small piece of cardboard. The cotton and cardboard were removed at the end of the day and the eggs allowed to hatch. On the morning after hatching the larvae were washed from the cardboard with distilled

water and about 50 larvae were pipetted into each individual test jar which contained approximately 100 grams of the feces.

The tests were evaluated on the basis of emergence. A 5-day development period was allowed for face fly larvae. At the end of this time the test material was carefully broken apart and all pupae removed and held for emergence. Five days after emergence began pupae that had failed to emerge were recorded. The house fly tests were conducted similarly except that 10 days were allowed for the larvae to develop. The emergence percentages given in table 1 for face flies are actual values; however, in the house fly tests the percentages are estimated on the basis of 55 larvae per jar. The house flies emerging in the pretreatment and posttreatment tests and in the controls indicated a low of 41, a high of 68, with an average of 54.6 flies per jar.

RESULTS AND DISCUSSION.—The results of the two experiments are presented in table 1. Tests were conducted several days prior to the feeding periods in both experiments to show that face flies and house flies would develop in the droppings of the animals. Since the pretreatment and final posttreatment data were essentially the same, the pretreatment results were omitted from the table. Face fly larvae were susceptible to all of the insecticides at both dosage levels. With one exception, the house fly larvae failed to develop in the manure of aimals fed the high dosage. The exception occurred with an animal that refused to eat all of the ronnel ration. On the first day of feeding, this animal ate less than 1 pound of the ration, and a fecal sample taken about 18 hours after the food was offered showed practically no toxicity. This animal did consume an overall daily average of 2.72 pounds during the feeding period; after the first test this was sufficient to completely inhibit the development of face fly larvae. However, some of the house fly larvae developed in every test. It is of interest to note that in the second experiment this same animal was placed on the Bayer 22408 ration and consumed a daily average of 3.9 pounds. Except for the one animal that disliked the ronnel ration, none reacted unfavorably to the feed additives.

The second experiment at lower dosages indicated that face fly larvae were more susceptible than house fly larvae to insecticide-contaminated manure. Ronnel at 2.5 milligrams per kilogram appeared to be near the minimum dosage for effective face fly control. Although all the larvae were killed at this dosage, examination of the test samples showed that many survived until they were almost fully grown. Inspection of the samples from animals treated with Co-Ral and Bayer 22408 showed that the face fly larvae were killed while they were very small. In the house fly tests, only the Co-Ral samples gave rapid larvicidal action, but examination of test material from the 0.5-milligram-per-kilogram dosage showed that many of the larvae survived until they were about half grown.

These results indicate that effective face fly control can be obtained when low dosages of Co-Ral, ronnel, or Bayer 22408 are added to the ration. However, extensive field trials are necessary to determine minimum dosages and the most practical means of administering an effective daily dosage. In the case of lactating dairy animals, further study is needed to determine whether such compounds would constitute a residue hazard in milk.

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Toxicity to Fly Larvae of the Feces of Insecticide-Fed Cattle[1]

GAINES W. EDDY and A. R. ROTH, *Entomology Research Division, Agric. Res. Serv., U.S.D.A., Corvallis, Oregon*

ABSTRACT

Studies were conducted on the toxicity to newly hatched house fly larvae (*Musca domestica* L.) of the feces of cattle fed 25 different compounds. The materials were incorporated in the animals' diet for 5 days and fecal samples collected for tests at different intervals during feeding and after it was discontinued. Fourteen of the 25 compounds were not completely lethal to the larvae at the dosages tested. Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate) and Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), the two most effective materials, were lethal to the larvae when administered to the animals at dosages as low as 1 mg./kg. Comparative data are presented on the susceptibility of newly hatched larvae of the house fly, stable fly (*Stomoxys calcitrans* (L.)), and horn fly (*Siphona irritans* (L.)) to four of the more effective insecticides.

Several important insects breed in the droppings of cattle. They include the house fly (*Musca domestica* L.), stable fly (*Stomoxys calcitrans* (L.)), and the horn fly (*Siphona irritans* (L.)). The discovery of the face fly (*Musca autumnalis* DeGeer) in North America by Vockeroth (1953) added another serious livestock pest to this list.

The control of feces-breeding insects through internal

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treatment with chemicals of cattle has not been investigated thoroughly. Studies by Knipling (1938) showed that the feces of cattle fed phenothiazine were toxic to larvae of the horn fly and Bruce (1942) found zinc oxide to be effective. Little further work along this line was done until that reported by Eddy *et al.* (1954). These authors showed that certain chlorinated hydrocarbon insecticides passed through cattle in a form toxic to dipterous larvae. Studies such as those by Robbins *et al.* (1957) on the metabolism of phosphorus compounds in cattle have shown that this class of insecticides is also excreted in the feces when fed or administered orally. The development of new and more effective insecticides during the last few years and our increased knowledge about them, especially with regard to their fate in cattle, encouraged the authors to undertake further studies during the fall of 1959 on the possible control of feces-breeding flies through the addition of insecticides to the animals' diet. This paper presents results obtained in preliminary tests with 25 compounds.

MATERIALS AND METHODS.—*Test Compounds.*—In selecting materials and the initial dosage rates to be tested, use was made of available information on larvicidal and mammalian toxicity, and metabolism in animals, especially livestock. Although many different materials were investigated, those such as dieldrin, DDT, and others known to be heavily stored in the tissues of animals were not included.

The materials tested that have no accepted common names or that are not properly identified in the tables are as follows:

Bayer 21/200—*O*-(3-chloro-4-methylumbelliferone) *O,O*-dimethyl phosphorothioate
Bayer 22408—*O,O*-diethyl *O*-naphthalimido phosphorothioate
Bayer 29493—*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate
Bayer 37342—*O,O*-dimethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate
Butonate—dimethyl 1-butyryloxy-2,2,2-trichloroethyl phosphonate
Co-Ral*—*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate
Diazinon*—*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate
Dipterex*—dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate
Dition*—*O,O*-diethyl *O*-(3,4-tetramethylene umbelliferone) ester of phosphorothioic acid
Dow ET-15—*O*-methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate
GC-4072—2 chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate
Hercules 4580—*S*-ethoxycarbonylmethyl *O,O*-diethyl-phosphorodithioate
Hercules 5727—*m*-isopropylphenyl methylcarbamate
Potosan*—*O,O*-diethyl *O*-(4-methylumbelliferone) phosphorothioate
Ryanex (*Ryania speciosa* Vahl)
Sevin*—1-naphthyl *N*-methylcarbamate

Experimental Animals and Feeding of Insecticides.—A majority of the cattle were yearling dairy-type animals purchased at a local auction. They were given untreated feed for 2 or more days prior to the addition of the chemical. During the tests the animals had access to fair grazing and a reduced amount of alfalfa hay.

The desired amount of the technical grade of the insecticide in 50 ml. of acetone was added to 2 or 3 pounds of a commercial cattle feed. Freshly prepared feed was given daily for 5 days. Some of the animals refused to eat part or all of the contaminated feed. These were given the chemical in a capsule with the aid of a balling gun.

Collection and Bioassay of Fecal Samples.—The number of fecal samples taken differed with the material and dosage rate being tested although a sample was always taken just before the first feeding. In initial tests with most of the materials, samples were taken on the fifth day and again 2 days after feeding was discontinued. In subsequent tests with materials that proved effective, samples were taken on the first and/or third and fifth days of feeding and at one or two intervals after feeding was discontinued. Each sample weighed 150 grams. Duplicates were run except in the exploratory experiments. They were placed inside pint glass jars 10 cm. in height and 7.5 cm. in diameter equipped with screen lids that contained a 100-ml. beaker full of wood shavings screened through a ¼-inch mesh. Fifty milliliters of shavings were placed on top of the feces following larval infestation. Materials which failed in initial tests were not examined further. Effective materials were fed to two or more animals.

The house fly was used as the principal test insect because of its availability, ease of handling, and the time of year in which the tests were conducted. Each sample was infested with approximately 50 newly hatched larvae. The house fly and stable fly larvae were from insecticide-susceptible laboratory colonies whereas the horn fly larvae were from field-collected specimens. Following the emergence of house flies, the adults were counted, sexed, transferred to screened cages measuring about 3×4×10 inches, and given food and water. Egging was done about 1 week after emergence to determine fertility. All tests of fecal samples were run at 80° F. and 60% to 65% relative humidity.

Laboratory Tests of Materials Added to Feces.—Three of the more effective materials in the animal tests were evaluated in the laboratory against newly hatched larvae of the house, stable, and horn fly by mixing them in feces from untreated dairy cattle. The desired amount of insecticide in 1.5 ml. of acetone was slowly added by pipette to 150 grams of feces while they were being stirred with an electric mixer. Some further mixing was done with a spatula. No shavings were added to the bottoms of the jars, but at 2 to 3 days after infestation a 50-ml. beaker full of shavings was placed on top of the feces to aid in pupation. Other conditions of the tests were the same as those described for feces from treated animals. Three to five replications were run with each material against each insect.

RESULTS AND DISCUSSION.—*Materials Fed to Animals.*—The data on the toxicity to house fly larvae of the feces of cattle fed different insecticides are summarized in table 1. These and other data not shown in the table indicated that Bayer 22408 and Co-Ral were the two most effective compounds, although the difference between them and some of the others was not great. Several of the materials, such as Diazinon (Robbins *et al.* 1957), would have no doubt proved effective had a greater amount been given.

Table 1.—Toxicity to house fly larvae of the feces of cattle fed or administered different compounds for a 5-day period.[a]

Insecticide	Larval Toxicity[b] at Indicated dosage—(mg./kg.)	
	Sublethal	Lethal
Bayer 22408	0.25	1.0
Co-Ral	.25	1.0
Bayer 21/200	.25	5.0
Potosan	.25	5.0
Dow ET-15	1.0	5.0
Ronnel	1.0	5.0
Dition	1.0	10
Gen. Chem. 4072	1.0	10
Bayer 37342	2.5	10
Parathion	5	10
Rotenone	10	25
Diazinon	2[c]	
Bayer 29493	5	
Dicapthon	5	
Dipterex	10	
Hercules 5727	10	
Ryanex	10	
Sevin	20	
Malathion	20	
Allethrin	25	
Barthrin	25	
Butonate	30	
Hercules 4580	50	
Diphenylamine	50	
Phenothiazine	50	

[a] Fifty larvae per sample.
[b] Sublethal: animal dosage allowing at least some fly emergence; lethal: dosage allowing no fly emergence.
[c] No higher dosages tried with this and following compounds.

Further tests were made with Bayer 22408, Co-Ral, and Dition to determine their relative effectiveness against larvae of the house, stable, and horn fly. In these tests the materials were also incorporated in the animals' diet and fecal samples taken on the third and fifth day of feeding. The data are presented in table 2. As expected, Bayer 22408 and Co-Ral were more effective than Dition and all three compounds appeared to be more effective against stable fly than against either house fly or horn fly larvae. Co-Ral appeared to be the most toxic to horn fly larvae.

Materials Added to Feces.—Three of the effective compounds, Bayer 22408, Co-Ral, and ronnel, were further evaluated in experiments whereby they were mechanically added to fresh cattle feces and these infested with the different larval species. The results are presented in table 3. The materials were about equally effective against stable fly larvae. However, Co-Ral was the most effective against horn fly larvae and ronnel the least effective against house fly larvae.

Table 2.—Larval toxicity as indicated by number of flies emerging from feces of cattle fed insecticides for 5 days.[a]

Insecticide	Dosage (mg./kg.)	Number Emerging from Samples Collected on Indicated Day of Treatment					
		House Flies		Stable Flies		Horn Flies	
		3rd	5th	3rd	5th	3rd	5th
Bayer 22408	0.05	29	31	1	0	18	25
	.25	0[b]	20	0	0	11	30
Co-Ral	.25	29	5	0	0	0	0
Dition	.25	54	31	8	—	31	21
	1.0	17	26	0	—	0	0

[a] Samples taken 3 and/or 5 days after initial dose. 50 larvae per sample.
[b] Not in agreement with 5th day or previous findings.

Table 3.—Comparative toxicity of materials to fly larvae when mixed in fresh cattle feces.[a]

Insecticide	Minimum Lethal Concentration in P.P.M. Indicated[b]		
	House Fly	Stable Fly	Horn Fly
Bayer 22408	0.25–0.5	0.25–0.5	0.5–1.0
Co-Ral	.25– .5	.25– .5	.1–0.25
Ronnel	.5 –1.0	.25– .5	.5–1.0

[a] Two to four tests, 50 larvae per sample.
[b] The first and second figures in each column represent sublethal and lethal concentrations, respectively.

Effect of Materials on Fly Fertility.—As indicated above, the flies from the treated feces were egged about 1 week after emergence to determine fertility. None of the materials appeared to influence egg viability, although a reduction in numbers was noted in some instances.

No special effort was made to obtain data on how long the insecticides remained effective after feeding was discontinued since most organophosphorus compounds are rapidly broken down and excreted by mammals (Plapp *et al.* 1960). The effective period would depend on several factors such as dosage, chemical stability, and/or metabolism of the insecticide by the animal. From the data obtained by the authors at least twice the effective dosage would be needed to give kill of insect larvae for as long as 48 hours after treatment was discontinued. For instance, Bayer 22408 was effective for 48 hours after treatment at 5 mg./kg. but not at 2.5 mg./kg., and Dow ET-15 was effective for 2 days after treatment at 20 mg./kg. but not at 10 mg./kg.

The effectiveness of Bayer 22408 and Co-Ral at such a low rate (1 mg./kg.) would clearly indicate that the control of feces-breeding fly larvae was not only possible but in the realm of practicability. Previous metabolic studies with these two compounds (Kaplanis *et al.* 1959, and unpublished data by Gatterdam, Chamberlain, Claborn, and others, USDA, Kerrville, Tex. 1959) have shown that the residue problem in livestock might not prove too serious at levels of 5 mg./kg. or less.

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Phorate Accumulation by Cotton Plants and Recovery from Soil[1]

JOSEPH HACSKAYLO,[2] D. A. LINDQUIST,[3] and J. C. CLARK[3]

ABSTRACT

Small-plot field tests were used to study phorate uptake by cotton plants following seed and in-furrow granular applications; phorate recovery from the soil was studied also. In-furrow granular treatments with phorate reduced phytotoxicity and gave aphid control similar to that with the standard carbon seed treatment. Studies with P^{32}-labeled phorate showed similar amounts of phorate-equivalents in plants treated by both methods.

Radioassay of soil cores showed 70% or more of the radioactivity in the top 1.5 inches 1 to 7 weeks after planting. The combined quantities of radioactivity extractable with chloroform and water decreased with time to a low of about 25% in each of the treatments. Generally, similar amounts of radioactivity were recovered in the chloroform and water extracts.

Use of phorate seed treatment for early-season control of cotton insects has not been widely accepted. The principal reason for this nonacceptance is that stand reductions often occur (Hanna 1956). Parencia *et al.* (1958) reported that emergence of reginned cotton seed, which contain damaged seed, was reduced by phorate seed treatment, whereas no reduction occurred when acid- or flame-delinted seed were similarly treated. Hacskaylo & Ranney (1957) reported that emergence of phorate-treated cotton seed planted in sand was comparable with that of untreated seed at 86° and 77° F.; however a marked reduction in emergence of treated seed resulted at 72°. Regardless of temperature, the rate of emergence was reduced by phorate seed treatment.

The small-plot field experiments reported herein were performed to establish whether in-furrow placement of phorate-impregnated granules would provide insect control comparable with seed treatment and whether the granular treatments would cause less phytotoxicity, as measured by seedling emergence, than the seed treatment. Also, phorate accumulation by field-grown cotton plants and recovery from the soil were studied.

MATERIALS AND METHODS.—*Seedling Emergence and Aphid Bioassay.*—This experiment was conducted in the field during the 1958 growing season. Because of the wide differences in specific gravity of the various granules used, it was thought that the granules should be equated on the basis of volume rather than on units of weight. A volume of 2.5 liters of 2/4- and 4/8-mesh ALVM attapulgite clay, 4/8- and 15/20-mesh vermiculite and 4/15-mesh carbon granules each was introduced into a 1-gallon wide mouth jar fitted with a screw-cap lid. Two liters of acetone containing 18.4 grams of 90% technical phorate were introduced into each jar and the jars tumbled for 1.5 hours. The acetone was evaporated and the jars tumbled again for 30 minutes. A conventional carbon dust-phorate seed treatment and untreated check also were included. Each treatment was replicated six times in a randomized-block design with each replicate consisting of 250 Deltapine-15 cotton seed planted in a 25-foot row. Two hundred fifty ml. of the treated granules (1.7 lbs. of phorate per acre) were sown immediately ahead of the seed. The seed treatment rate was 1 pound of phorate per acre.

Seedling emergence was observed daily for 10 days. The insecticidal effectiveness was estimated by natural infestations of the cotton aphid, *Aphis gossypii* Glov. Calcium arsenate-DDT dust was applied to the plants to aid aphid population increase.

Phorate Accumulation by Plants and Recovery from Soil.—To determine the fate of phorate in the soil and its accumulation by the plants, a second field test using P^{32}-labeled phorate was conducted during the 1959 growing season. The granules were handled as previously described and the treatments were as follows:

1. Seed treatment: 4 mg. P^{32}-labeled phorate per seed (1 lb. phorate per acre).
2. Attapulgite granules: 668 mg. P^{32}-labeled phorate was added to 100 ml. 24/48-mesh granules (1.7 lbs. phorate per acre).
3. Attapulgite granules plus deactivator: Same as 2 except for addition of 601 mg. of deactivator E[4] (1.7 lbs. phorate per acre).
4. Vermiculite granules: Same as 2 except for use of 15/20-mesh vermiculite granules (1.7 lbs. phorate per acre).

Each treatment consisted of 100 seeds and 100 ml. of the granules in a single 10-foot row in Lufkin fine sandy loam. The plot was watered to insure adequate growth of the plants.

Three to five plants from each treatment were collected 1, 2, 3, 4, and 6 weeks after planting and dried in an oven at 70° C. for 24 hours. The plants were then weighed and ground in a Wiley mill to pass a 60-mesh screen. Samples of 150 to 200 mg. of the plants were placed in 1-inch stainless steel planchets and radioassayed with a thin window Geiger-Meuller tube and a conventional scaler.

Duplicate soil cores 3 inches in diameter and 12 inches deep were removed from the center of the rows of treatments 1, 3, and 4; 2, 4, and 7 weeks after the planting

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[2] Plant Physiologist, Crops Research Division.

[3] Entomologist, Entomology Research Division.

[4] Supplied by American Cyanamid Co., Rockefeller Plaza, New York, N. Y.

Table 1.—Average cotton seedling emergence and percentage plants infested with aphids following phorate seed treatment and in-furrow granular applications.

Phorate Treatment	Seedling Emergence (%)	Plants Infested with Aphids at Indicated Weeks		
		10	11	12
		(%)		
None (control)	68	23	92	100
Seed treatment[a]	42	0	75	98
15/70-mesh Vermiculite[b]	66	2	42	85
4/8-mesh Vermiculite[b]	65	2	58	73
2/4-mesh Attapulgite[b]	65	4	65	81
4/8-mesh Attapulgite[b]	63	0	52	65
4/14-mesh carbon[b]	62	4	65	89

[a] Phorate applied at 1 lb. per acre.
[b] Phorate applied at 1.7 lbs. per acre.

date. The soil cores were divided into four sections (0 to 1.5 in., 1.5 to 3 in., 3 to 6 in., and 6 to 12 in.), dried in an oven at 80° C. for 24 hours and mixed thoroughly and 50-gram samples were removed. Two aliquots of each sample were radioassayed to determine the total radioactivity in the 50-gram samples. The soil was subsequently extracted three times with 100-ml. portions of chloroform, air-dried, and extracted once with 100 ml. of water. Bowman & Casida (1957) reported that phorate and its oxidative metabolites were recovered from plant brei with chloroform, whereas the hydrolytic products remained in the aqueous phase. It was assumed in this work that the chloroform fraction contained the phorate plus its oxidative metabolites while only the hydrolytic products were in the water extract. The radioactivity in the extracts was assayed with a solution counter which consisted of a thin glass-walled dipping tube mounted securely in a glass sample holder and a conventional scaling unit.

RESULTS.—*Seedling Emergence and Aphid Bioassay.*—Table 1 shows that only the phorate seed treatment caused a reduction in seedling emergence. All the granular treatments as well as the seed treatments gave 100% aphid control for a 9-week period following emergence, indicating that the plants absorbed phorate equally well whether the insecticide was applied as a seed treatment or in furrow granular formulations.

Phorate Accumulation by Plants and Recovery from Soil.—The accumulation of P^{32}-labeled phorate by cotton plants was comparable whether the insecticide was applied as a seed treatment or as in-furrow granular formulations (table 2). The addition of the deactivator to the attapulgite granules appeared to favor initial phorate accumulation by the plants as compared with the attapulgite alone and vermiculite granules. This increased absorption would be of no practical significance since a maximum of 5% to 7% of the applied dose was finally absorbed regardless of treatment.

The leaching of the radioactivity from Lufkin fine sandy loam was very slight regardless of exposure time (table 3). In all treatments, the greatest quantities were found in the top 1.5-inch layer of soil. Where phorate was applied as a seed treatment, rather uniform amounts of

Table 2.—Accumulation of P^{32}-labeled phorate equivalents by cotton plants following carbon seed treatment and in-furrow granular applications.[a]

Phorate Treatment	Micrograms Phorate Equivalents per Plant at Indicated Weeks After Planting				
	1	2	3	4	6
Seed treatment[b]	173	147	213	187	241
24/48-mesh Attapulgite granules[c]	123	133	122	181	222
24/48-mesh Attapulgite granules plus Deactivator[c,d]	142	148	210	235	322
15/20-mesh Vermiculite granules[c]	78	105	168	151	300

[a] Results expressed as averages of 2 to 5 plants.
[b] Phorate applied at 1 lb. per acre.
[c] Phorate applied at 1.7 lbs. per acre.
[d] See footnote 4.

the insecticide were leached to the lowest regions sampled. The recovery of the radioactivity from the 1.5- to 3-inch depth in the granular treatments was considerably greater than from the two lower depths, but still less than from the 0 to 1.5-inch layer. This increased recovery of radioactivity from the 1.5- to 3-inch depth was undoubtedly because of the placement of the granules in the seed furrow.

In general more radioactivity was recoverable in the chloroform than in the water extracts of soil cores from the seed treatment (table 4). The percentage total extractable radioactivity decreased with time, and would tend to indicate that products other than phorate are being continually tied up by the soil particles. This same trend was observed for the vermiculite granules.

It is noteworthy, however, that the quantity of radioactivity extracted from the soil of the attapulgite plus deactivator treatment was greater in the water than in the chloroform phase. The per cent total extractable radioactivity decreased with time, as was found in the other two treatments.

DISCUSSION.—The insect-control data and the P^{32}-labeled phorate-accumulation data show that comparable

Table 3.—Vertical distribution of P^{32}-labeled phorate detected in 50-gram samples at indicated soil depths and time intervals.

Phorate Treatment	Soil Depth (in.)	Per Cent Total Radioactivity at Indicated Weeks After Planting		
		2	4	7
Seed treatment[a]	0.0–1.5	94	75	71
	1.5–3.0	4	4	8
	3.0–6.0	1	10	13
	6.0–12.0	1	10	8
24/48-mesh Attapulgite granules plus Deactivator[b,c]	0.0–1.5	69	76	58
	1.5–3.0	29	17	39
	3.0–6.0	1	5	2
	6.0–12.0	1	2	1
15/20-mesh Vermiculite granules[c]	0.0–1.5	76	80	79
	1.5–3.0	20	14	15
	3.0–6.0	3	4	3
	6.0–12.0	1	2	3

[a] Phorate applied at 1 lb. per acre.
[b] See footnote 4.
[c] Phorate applied at 1.7 lbs. per acre.

Table 4.—Extraction of radioactivity from soil treated with P^{32}-labeled phorate as a seed treatment and in-furrow granular treatments for cotton insect control.

Phorate Treatment	Soil Depth (in.)	Phorate-Equivalents Extracted at Indicated Weeks After Planting								
		2			4			7		
		$CHCl_3$ μg.	H_2O μg.	Both (%)[a]	$CHCl_3$ μg.	H_2O μg.	Both (%)[a]	$CHCl_3$ μg.	H_2O μg.	Both (%)[a]
Seed treatment[b]	0.0– 1.5	150	307	63	76	26	47	63	47	26
	1.5– 3.0	14	11	92	6	2	100	13	10	46
	3.0– 6.0	8	3	100	16	6	100	17	4	30
	6.0–12.0	4	3	78	6	4	37	8	4	15
24/48-mesh Attapulgite granules plus Deactivator E[c,d]	0.0– 1.5	337	399	55	76	76	32	56	138	21
	1.5– 3.0	115	177	51	15	17	32	50	80	21
	3.0– 6.0	7	0	26	6	9	60	0	7	23
	6.0–12.0	1	1	100	0	1	3	0	5	20
15/20-mesh Vermiculite granules[d]	0.0– 1.5	340	217	59	572	265	64	265	134	25
	1.5– 3.0	73	101	69	70	46	48	50	33	32
	3.0– 6.0	2	5	24	27	13	60	12	5	6
	6.0–12.0	3	0	23	2	7	35	0	6	25

[a] Based on total radioactivity in 50 grams dry soil.
[b] Phorate applied at 1 lb. per acre.
[c] See footnote 4.
[d] Phorate applied at 1.7 lbs. per acre.

amounts of phorate are accumulated by plants from both seed and in-furrow granular treatments. It was evident also that granular formulations were less phytotoxic than the seed treatment method since increased emergence was observed in the former. Lindquist *et al.* (unpublished data) found that following seed treatment, most of the absorbed insecticide was absorbed during the germination process. In-furrow granular applications would tend to eliminate most of the direct contact of the insecticide with the seed, and for the most part would permit insecticide accumulation after rather than during germination. Unfortunately, however, approximately 95% of the applied dose goes unused by the plants regardless of the method of application.

Less than 30% of the radioactivity found in all treatments was recovered below the 3-inch soil depth even at the 7-week sampling time. Getzen & Chapman (1960) reported similar results for leaching of phorate from a loam type soil under laboratory conditions. Also, these authors showed that increased quantities of radioactivity were bound to the soil with time as reported here. On the other hand, our results show large quantities of radioactivity in the water extract, whereas their data show 7% or less as hydrolytic products. Since the results reported by Getzen & Chapman (1960) were obtained from soils held in shell vials in the laboratory, it is possible that the degradation pattern of the insecticide would be altered as compared with the field experiment reported herein. Also, the soil types used could possibly produce the above-noted difference.

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Aphid Transmission of Bean Viruses in Field Beans in Central Washington[1]

RICHARD S. ROBERTSON, JR.[2] and E. C. KLOSTERMEYER, *Washington State University, Pullman*[3]

ABSTRACT

Epidemiology studies of bean mosaic diseases in central Washington were made from 1955 to 1959. Surveys of aphid populations and mosaic disease symptoms were made in plots of field beans. Aphids rarely colonized the bean plants, but moderate to large populations of alate aphids were present from late June until sometime in August. The majority were green peach aphids, *Myzus persicae* (Sulzer). The bulk of the remainder consisted of pea aphids, *Macrosiphum pisi* (Harris), and English grain aphids, *Macrosiphum granarium* (Kirby). Possible arthropod vectors other than aphids were very few. Mosaic symptoms began appearing in the bean plots in July and were well distributed by early August. A number of different nonpersistent viruses were isolated and subjected to greenhouse tests. All were readily transmissible by the green peach aphid and somewhat less so by the clover aphid, *Anuraphis bakeri* (Cowen). None were transmitted by the English grain aphid. The pea aphid transmitted these viruses only from broadbean. It was concluded that virus spread in the bean fields was due to migrant aphids, especially the green peach aphid, and, because of the short transmission feeding times, vector control methods would likely be of little value in controlling the mosaic diseases.

The relationship between aphid populations and spread of mosaic diseases was studied in plots of field beans at the Irrigation Experiment Station in south central Washington's Yakima Valley during the summers of 1955, 1956, 1957, and 1958. Correlated greenhouse studies of the aphid transmission of these mosaics were made at the main Experiment Station, Pullman, between 1955 and 1959.

APHID POPULATIONS OF FIELD BEANS.—Robertson & Klostermeyer (1958) reported in detail on the results of the aphid population studies of 1955 and 1956. The majority of aphids collected during these studies were alate green peach aphids, *Myzus persicae* (Sulzer). Other aphid species collected included the English grain aphid, *Macrosiphum granarium* (Kirby), and the pea aphid, *Macrosiphum pisi* (Harris), plus smaller numbers of several other species. The aphids were usually unable to colonize on the bean plants. Aphid population peaks in these bean fields occurred at the end of July in 1955 and in mid-July in 1956.

In 1957, field studies were made in a 2-acre field planted in three varieties of field beans in a randomized block design of eight replications. The varieties were: Early Pink, Red Mexican U.I. #34, and Pinto U.I. #111. Collections of aphids were made from 25 leaves per plot on July 17 and July 31. Nearly all of the aphids collected were alatae. On July 17, of 514 alate aphids collected from 600 leaves, 390 (75.9%) were green peach aphids. On July 31, 600 leaves yielded 1,165 winged aphids, 1,071 (91.9%) of which were green peach aphids. No significant variations in aphid populations were observed on the different bean varieties.

In 1958, field studies involved the above three bean varieties plus Columbia Pinto and Great Northern U.I. #31 in a randomized block design of eight replications. Six aphid collections were made, each from 25 bean leaves per plot. Alatae collected from all varieties (1,000 leaves) numbered 12 on June 9, 77 on June 24, 90 (75 green peach aphids) on July 1, 40 on July 15, 50 on July 22, and 12 on August 5. Cowpea aphids, *Aphis medicaginis* Koch, and English grain aphids were most abundant in the June collections. A few pea aphids were present in all collections. Green peach aphids were also in all collections.

Peak aphid collections for each year from the leaves of Early Pinks and Red Mexican U.I. #34 are compared in table 1. More aphids were collected from the Early Pinks than from the Red Mexican variety. About 70% of these aphids were green peach aphids.

Table 1.—Alate aphids collected from leaves of two bean varieties, Prosser, Washington, 1955–1958, peak collection for each year.[a]

Date	Early Pink			Red Mexican U.I. #34		
	Myzus persicae	Other Species	Total	*Myzus persicae*	Other Species	Total
July 21, 1955	404	505	909	473	314	787
July 16, 1956	747	106	853	263	65	328
July 31, 1957	376	38	414	264	21	285
July 1, 1958	18	2	20	11	1	12
Total	1,545	651	2,196	1,011	401	1,412
Percentage	70.3	29.7	100.0	71.7	28.3	100.0

[a] Number of aphids per 200 leaves for each date and variety.

Aphid collections for July 17, 1957, included one alate specimen of *Macrosiphum gaurae* Williams, previously unreported from the Yakima Valley.

DISEASE SURVEYS IN FIELD BEANS.—These surveys were made by examining 25 plants in each plot. Virus symptoms observed in the bean fields were usually scattered haphazardly, rather than confined to specific areas within each field. This suggested involvement of winged, rather than wingless, vectors.

The 1955 surveys were conducted in fields planted in five bean varieties: Early Pink, Red Mexican U.I. #34, Pinto U.I. #72 and #111, and Great Northern U.I. #16. Mosaic symptoms were first noted on July 27. These symptoms were found in many plants by August 15 when a count was made. Symptoms were classified into two main categories at this time. Yellow mosaic-type symptoms consisted of a vein necrosis and/or a severe foliage mottling and dwarfing. Common mosaic-type symptoms consisted of a mild mottling and/or a pronounced leaf

[1] Most of these data are taken from a thesis submitted to the Graduate School of Washington State University, Pullman, Washington, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Scientific Paper No. 2009, Washington Agricultural Experiment Stations, Pullman, Project 1221. Cooperative Regional Research Project W-12. Accepted for publication November 3, 1960.

[2] Now with the Department of Science Education, San Jose State College, San Jose, California.

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Table 2.—Percentages of bean plants displaying mosaic symptoms, Prosser, Washington, 1955–58.

Symptom Type	Bean Variety: Early Pink	Red Mexican	Pinto UI-72	Pinto UI-111	Great Northern
A. August 15, 1955					
Common mosaic	1.5	38.5	12.0	17.0	0.0
Yellow mosaic	96.5	16.0	66.5	52.0	86.5
Both common and yellow	2.0	6.5	5.0	11.5	0.0
Other symptoms	1.5	1.5	3.5	2.0	0.0
No symptoms	4.5	37.5	13.0	17.5	13.5

Symptom	Bean Variety: Early Pink	Red Mexican
B. August 10, 1956		
Vein necrosis only	31.5	18.0
Necrosis and mottle	60.0	53.5
Mottle only	5.0	17.5
Necrosis and epinasty	2.5	0.5
No symptoms	1.0	10.5

Symptoms	Bean Variety: Early Pink	Red Mexican	Pinto UI-111
C. August 13, 1957			
Vein necrosis only	32.5	1.5	6.5
Necrosis and mottle	63.5	9.0	23.0
Mottle only	0.0	43.5	19.0
Necrosis and epinasty	1.5	0.5	1.5
Epinasty only	0.0	0.5	1.5
No symptoms	2.5	45.0	48.5

Symptoms	Bean Variety: Early Pink	Red Mexican	Pinto UI-111	Great Northern	Columbia Pinto	Special Strips[a]
D. August 6, 1958						
Vein necrosis only	23.0	4.5	11.0	13.5	17.5	18.0
Necrosis and mottle	60.0	10.0	15.5	0.0	19.0	36.0
Mottle only	8.0	13.0	9.5	0.0	15.5	24.0
No symptoms	9.0	72.5	64.0	86.5	48.0	22.0

[a] Rows of Columbia Pintos planted with seed from a field badly infected with seed-transmissible "D" virus.

cupping, with some reduction in foliage size. The results are given in table 2,A.

The 1956 surveys involved Early Pink, Red Mexican U.I. #34, and Columbia Pinto bean varieties. Mosaic symptoms began appearing in the Pink and Mexican fields soon after mid-July and were widely distributed before mid-August. Less than 1% of the Pinto plants ever developed mosaic symptoms. Results of a survey of mosaic diseases in the Early Pink and Red Mexican bean fields made on August 10 are presented in table 2,B. Three types of symptoms are listed: veinal necrosis, foliage mottling (usually with dwarfing), and epinasty.

The 1957 surveys were made in the field already described under "Aphid Populations on Field Beans." Mosaic symptoms began to appear in late July and were widespread by mid-August, as indicated in table 2,C.

The 1958 surveys were made in the experimental field described earlier in this paper. In four of the Columbia Pinto blocks of this planting, two of the eight rows were planted with seed from a field badly infected with the "D" virus described below. Mosaic symptoms began appearing in late June. Symptom surveys made on August 6 are summarized in table 2,D. By the end of August, mosaic symptoms were present in approximately 95% of the Early Pink bean plants, 50% of the Red Mexicans, 40% of the Pinto U.I. #111, 75% of the Columbia Pintos, and none of the Great Northerns.

APHID TRANSMISSION TESTS.—Greenhouse tests to determine the efficiency of certain aphid species in transmitting the viruses involved the rearing of aphids on virus-free plants, starving of adult aphids for a period of 1 to 1-½ hours, placing them on a mosaic-infected plant for approximately 5 minutes, and then transferring them to young healthy test plants for a period of 1 hour, five aphids per plant. This technique was developed and used by Watson (1938, 1946) and Severin & Tompkins (1948). It has proven highly effective in the transmission of nonpersistent plant viruses. The viruses used in this study were readily transmitted mechanically and were of the nonpersistent type.

Aphids were considered the most likely suspects as the virus vectors for two reasons. First, aphids have been found to be responsible for most of the spread of nonpersistent viruses, wherever the epidemiology has been worked out. Secondly, aphids were present in much larger numbers than any other possible vectors when the viruses were being spread in the field (symptoms for the viruses involved appear approximately 10 days after a susceptible bean plant has been inoculated, under summer growing conditions).

The aphid species used in virus transmission tests included the more abundant species found on beans (green peach aphids, pea aphids, and English grain aphids) as well as aphids which commonly breed on other legumes (pea aphids and clover aphids, *Anuraphis bakeri* (Cowen)). Species in this latter category have been found to be major vectors of some bean mosaics by many workers.

In greenhouse tests, using Red Mexican U.I. #34 beans as test plants, the common mosaic-type virus was transmitted by green peach aphids to 19 of 20 plants, thus verifying the insect transmission of this disease.

From virulent bean plants in the 1956 bean fields, Skotland (1957) isolated three viruses here referred to as Viruses "A," "B," and "C," all apparently distinct from previously described bean viruses. A fourth virus referred to as "D" was isolated in 1957 by Skotland & Burke (1958) from a field of Columbia pinto beans in Benton County, Washington.

Symptoms induced in beans by virus "A" were a spreading veinal necrosis originating at the point of inoculation and sometimes followed by a top necrosis in some bean varieties. A severe mottling and dwarfing was produced in later-developing bean foliage of several varieties tested. Virus "B" induced a mild foliage mottling in several bean varieties, with an occasional localized vein necrosis. Virus "C" produced a mild foliage mottling, typically more severe than that induced by virus "B." Occasional localized veinal necrosis also resulted. The "D" virus was seed transmitted and produced symptoms suggestive of common bean mosaic but also sometimes resulted in the rusty colored veinal necrosis produced by the other three viruses. The symptoms of the four isolates were distinct when compared on a single bean variety but probably cannot be differentiated under field conditions.

The comparative ability of the four aphid species to transmit the four viruses is indicated in table 3. Each of the viruses was transmitted by the green peach aphid and by the clover aphid, but not by the English grain aphid which is found on beans only as a winged migrant. The pea aphid transmitted the viruses in these tests only when broadbean was used as a virus source. Adlerz (1959) noted that a higher rate of bean yellow mosaic transmis-

Table 3.—Summary of aphid transmission tests of mosaic viruses to Early Pink bean plants, 1957–1959.[a]

Aphid Species	Virus Source	Virus "A"	Virus "B"	Virus "C"	Virus "D"
Green Peach aphid	Early Pink	—	—	—	37/50
	Sweetclover	150/200	63/150	119/150	—
Clover aphid	Early Pink	—	17/50	7/50	11/50
	Sweetclover	11/50	—	—	—
Pea aphid	Early Pink	0/50	0/50	0/50	—
	Sweetclover	0/100	0/50	0/50	—
	Broadbean	18/50	2/49	4/50	20/50
English grain aphid	Early Pink	0/50	0/50	0/50	—

[a] Numerator indicates number of plants infected; denominator indicates number of plants tested. Five aphids were used per plant.

sion by pea aphids and green peach aphids occurs when the virus source plant is broadbean rather than beans.

In addition to these tests, the green peach aphid transmitted "A" virus to Red Mexican U.I. #34 and Pinto U.I. #111 bean varieties, to red clover and to sweetclover. The "B" virus was transmitted to Red Mexican & Pinto beans and to sweetclover. The "C" virus was transmitted to Red Mexican, to Pinto, to sweetclover, and to broadbean by this aphid.

These studies clearly indicate the importance of aphids in the field spread of these bean mosaics. The green peach aphid, usually an abundant vector, was probably responsible for most of this mosaic spread, but the pea aphid and the clover aphid may also serve to introduce the viruses into the bean fields from other legume hosts on which they breed.

No attempt can be made to say that specific amounts of specific viruses were present in the fields studied. Zaumeyer & Thomas (1957), and many other investigators, as well as the authors of this paper have noted the extreme variability of symptoms caused by any one kind of virus in any one species and variety of host plant. All of the mosaic symptoms observed in the field were at times seen in greenhouse bean plants infected with any of the viruses "A," "B," "C," and "D." However, the vein necrosis symptom was much more commonly associated with virus "A" than with any of the other viruses.

It is notable that, while the aphid populations varied considerably during the 4 years of the field surveys, the incidence of mosaic in any one bean variety varied little. On the other hand, the variation in incidence of mosaic diseases among the different varieties was considerable. Early Pink variety apparently was the most susceptible.

APHID ACTIVITY.—Kennedy & Stroyan (1959) reviewed the findings on aphid migration and host-finding. They concluded that host-finding by aphids is a hit-or-miss proposition. This results in aphids feeding on plants on which they do not breed. As Tate (1940) and Dickson *et al.* (1949) discovered, this habit enables aphids to transmit virus diseases to and from plant hosts on which those aphids do not breed.

Kennedy (1950) noted that migrant green peach aphids are especially restless and prone to move about. Our observations on individual green peach aphids, plus the data already here reported, agree with the above findings.

APPLICATIONS.—Broadbent (1957) has reviewed attempts to control plant virus diseases by vector control. He concluded that successful chemical control of nonpersistent virus diseases would usually require extremely fast-acting insecticides. The present study, involving extensive transmission of nonpersistent virus by migrant aphids (particularly the efficient and active green peach aphid), indicates that Broadbent's thesis would apply here.

At present, the best hope of controlling mosaic diseases in beans appears to lie in other directions, such as breeding varieties resistant to these viruses. Promising work along this line is being done by Baggett & Frazier (1959). They discovered that the runner bean, *Phaseolus coccineus* L., is immune to all bean viruses to which it has been subjected, including the viruses "A," "B," and "C" referred to as "node necrosis," "mottle," and "pod blotch," respectively.

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A Comparative Study of Certain Biological Phenomena of a Resistant and a Susceptible Strain of the Boll Weevil, *Anthonomus grandis*[1]

JOHN G. THOMAS[2] and J. R. BRAZZEL[3]

ABSTRACT

Research was conducted in 1959 to determine if biological differences existed in a laboratory reared resistant and susceptible strain of the boll weevil, *Anthonomus grandis* Boh.

The most significant differences found were an increase in the length of the developmental period and a decrease in the reproductive potential of the resistant strain. An increase of 12.5 hours in the developmental period of the resistant strain over the the susceptible strain proved highly significant. The fecundity of the resistant females decreased approximately 22% below the susceptible females.

Significant differences were observed in the fecundity, and the length of time required for embryonic, larval, and pupal development. No differences were observed in the mortality rates, sex ratios, length of preoviposition and oviposition periods or percentage egg hatch.

The most spectacular consequence of repeated use of several newer insecticides has been the development of resistance to them by some insects. This phenomenon has been under investigation at many locations in recent years. Emphasis has been placed on the study of resistance to chlorinated hydrocarbon insecticides by the boll weevil, *Anthonomus grandis* Boh., following its discovery by Roussel & Clower (1957) in Louisiana. Comparisons of the life history of resistant and susceptible strains have been made by many research workers to determine if other biological functions were affected by resistance and to offer some explanation of how resistance developed.

Many life history studies on the boll weevil have been made since this pest entered the Cotton Belt of the United States in 1892 (Coad 1915, Fenton & Dunnam 1929, Hunter & Hinds 1904, Smith 1921 and others). These workers were handicapped by the rearing techniques in making accurate records of the length of various stages in the developmental cycle. To observe the development of the immature boll weevil it was necessary to cut into the developing cotton fruit, thus disturbing the natural environment of the developing larva and pupa. Fenton & Dunnam (1929) and others pointed out that subjecting the immature stages to desiccation, changing temperature, and other external forces resulted in extreme variations in the required length of time for development and often resulted in death.

Hunter & Hinds (1904), Fenton & Dunnam (1929), Smith (1921), and Coad (1915) noted variations in results recorded from year to year and in generations within any given season. These variations were explained by differences in the temperature, humidity, rainfall, soil and condition of the cotton plant.

The recent development of boll weevil mass-rearing techniques on artificial diets (Brazzel *et al.* 1959, Vanderzant & Davich 1958) provides the researcher an opportunity to make detailed, accurate observations under controlled laboratory conditions. These techniques have made possible a more critical study of the biology of this insect.

It is generally believed that resistance occurs as the result of the exposure of insect populations to insecticidal selection pressure (Crow 1957, King 1954, Brown 1958). Since the establishment of boll weevil resistance to chlorinated hydrocarbon insecticides biological studies of this insect have again become important in determining whether differences exist in resistant and susceptible strains.

It has been noted that the number of individuals resistant to certain insecticides is extremely small prior to applying insecticidal pressure. Hunter (1956) stated: "Phenotypic differences between resistant and susceptible *Drosophila* should be expected if the genes causing resistance also affect other measurable characters directly by pleiotropism, or indirectly by linkage with genes controlling other morphological or physiological traits."

Several workers believe that the genotype of resistance is linked with genes for characters which are detrimental to the population (Pimental *et al.* 1951, Babers *et al.* 1953, Johnston *et al.* 1954, Grayson 1954, Crow 1954, King 1954). Extensive biological, physiological, and morphological comparisons have been made between resistant and susceptible strains of the house fly (*Musca domestica* L.), vinegar fly (*Drosophila* sp.), German cockroach (*Blattella germanica* (L.)), mosquitoes, and others.

Pimental *et al.* (1951), Hunter (1956), McKenzie & Hoskins (1954), Decker & Bruce (1952), Johnston *et al.* (1954) and others reported an increase in the length of time required for larval and pupal development associated with resistance in house flies. However, Babers *et al.* (1953), March & Lewallen (1950) and Keiding & Boggild (1957) found no differences in the time required for immature development, or the susceptible strain required a longer developmental period.

Comparative house fly investigations of the cuticular pigmentation, tarsal bristles, tarsal segments, cuticular and intersegmental membranes, susceptibility to immobility by heat and cold, ability to metabolize DDT to DDE, effects of crowding larvae in the media, weights of the various stages and reproductive potential have been made by many researchers with varying results. Variability in these characters has been attributed to the differences in the components or makeup of the population's gene-pool (Crow 1954, King 1954, Pimental *et al.* 1951). Crow (1957) pointed out that many instances of morphological, physiological, enzymatic, and behavioral differences between resistant and susceptible strains were probably of no significance, but were properties of the particular strains studied and not associated with resistance.

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[2] Assistant Entomologist, Texas Agricultural Extension Service. Research conducted in partial fulfillment of the requirements of Master of Science Degree in Entomology.

[3] Associate Professor, Texas Agricultural Experiment Station.

Table 1.—Comparison of the average length of embryonic, larval, pupal, and total developmental periods in a resistant and susceptible strain of the boll weevil.

Stage or Sex	Resistant		Susceptible		L.S.D. at 5% Level
	No. Observed	Average Length (days)	No. Observed	Average Length (days)	
Eggs	4,061	3.1	4,502	3.0	0.007
Larvae	1,932	8.5	1,934	8.4	.06
Pupae	1,932	5.0	1,934	4.7	.10
Average Total Developmental Period					
Males	1,205	16.6	1,225	16.0	0.12
Females	1,118	16.6	1,134	16.2	.12
L.S.D. at 5% level		.13		.11	
Total or mean	2,323	16.6	2,359	16.1	0.09

Comparisons between resistant and susceptible boll weevils were made by Bielarski *et al.* (1957) and they reported that no significant differences were found in the length of the developmental stages or reproductive potential of the two strains. They stated that since no differences in the two strains were detected, strong evidence pointed to the fact that the selection for resistance in the population was carried on independently of inheritance for other biological characters.

METHODS AND MATERIALS.—Resistant and susceptible stock cultures of newly emerged weevils were placed in 8″×8″×8″ plastic screen cages with a wooden bottom and glass front. Each stock culture consisted of 50 males and 50 females. The resistant and susceptible strains used in these investigations originated from a common parental stock collected in Mexico in 1957 (Brazzel & Lindquist 1959). The susceptible strain was not subjected to insecticidal pressure. The resistant strain was selected with endrin for 14 generations. The resulting level of resistance was between 75- and 100-fold compared with the unselected strain.

The stock cultures were used to insure an adequate number of eggs which were placed daily in an artificial diet. The mass-rearing techniques and larval diet described by Brazzel *et al.* (1959) were used.

The first stock cultures were prepared on June 12, and the progeny observed for a period of 30 days. The test was replicated beginning July 12 and the progeny observed for a period of 30 days. The first replication is designated resistant I and susceptible I and the second replication resistant II and susceptible II in the discussion of results.

Eggs were removed daily from both resistant and susceptible stock cultures and placed in the larval diet. The eggs were removed from marked cotton squares to allow no more than 4 hours variability in recording the time of oviposition. Developmental observations were made at 12-hour intervals.

All tests of fecundity were conducted by randomly selecting females from each strain daily. Each female was isolated in a plastic jelly dish measuring 95 mm. in diameter and 25 mm. deep. Two males of the same strain were placed with each female to insure fertilization.

By using progeny of a common parental stock, an artificial diet, and mass-rearing techniques under controlled laboratory conditions, it is believed that the differences that occurred between the two strains could be attributed to characters peculiar to resistance or susceptibility rather than to differences in genetic makeup, diet, temperature, humidity, et cetera. The data were analyzed by analysis of variance.

RESULTS AND DISCUSSION.—It was found that all eggs hatched between 48 and 108 hours after oviposition. The majority of the eggs in both strains hatched in 65 to 75 hours. The per cent egg-hatch was 92.38 ± 5.01 in the resistant strain and 94.62 ± 5.04 in the susceptible strain, not a significant difference.

The average length of embryonic development for weevils of each strain is presented in table 1. The difference in the two means was highly significant. It should be noted that the observed difference is 0.02 days (approximately 30 minutes); whereas, there is a possible variation of 4 hours in recording the time of oviposition.

The resistant strain required a longer average larval period, table 1. The comparison between the average time required for larval development in the two strains was highly significant.

The average length of the pupal stage in the resistant and susceptible strain is given in table 1. The greater length of time required for pupal development in the resistant strain was highly significant in both replications and in the overall means of the two strains.

The total time required for development from oviposition to the emergence of the adult is presented in table 1. More than 2,300 individuals in each strain were used to determine these means. These data indicate that the resistant strain required an average of 12.5 hours longer to complete development than the susceptible strain. The two strains of weevils were reared under controlled conditions, attempting to limit the variability to characteristics peculiar to each strain. If these two strains were characteristically capable of showing this much difference under controlled environmental conditions, it is possible that in the field a greater difference might be observed. This assumption is based on the belief that susceptible insect populations are better adapted to fluctuating environmental conditions; thus, they are predominant in the absence of insecticidal pressure (King 1954, Crow 1957).

In comparing the emergence of each strain it was found that the percentage of susceptible adults that had emerged daily during each test was greater than for the resistant strain, figures 1 and 2.

The average developmental period was determined for each sex within each strain (table 1). It was found that both sexes of the resistant strain required a significantly longer developmental period than the corresponding sex of the susceptible strain. There was no difference in the time required for development between the males and females of the resistant strain. However, the susceptible females required a significantly longer developmental period than the susceptible males. These findings indicate that the longer developmental period required by the resistant strain was the result of an increase in both female and male developmental periods.

There was no significant difference in the number of males and females emerging in each strain even though slightly more males than females were observed in both replications of each strain, table 1.

No significant differences were found in comparing the weights of the resistant and susceptible males and fe-

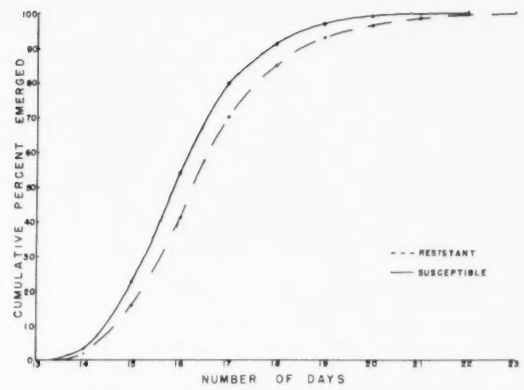

FIG. 1.—Length of developmental period of a resistant and susceptible strain of the boll weevil reared on a larval diet during the period June 14–July 14, 1959.

males. The females were significantly heavier than the males in both strains, table 2.

The average preoviposition and oviposition periods for the two strains did not differ significantly. The average preoviposition period for the resistant and susceptible strain was $4.01 \pm .148$ days and $4.18 \pm .125$ days, respectively. An average oviposition period of $11.73 \pm .898$ days for the resistant strain and $13.09 \pm .731$ days for the susceptible strain was observed.

The results of fecundity tests with isolated females are presented in table 3. It was found that the susceptible females averaged 31.7 more eggs per female than the resistant females. This difference was found to be significant at the 5% level, even though the data were extremely variable—as shown by the range in individual fecundity.

CONCLUSION.—As a result of these investigations it was determined that the developmental period was increased and fecundity decreased in the resistant strain, using the susceptible strain as a check or control. Since these two strains of the boll weevil were from a common parental

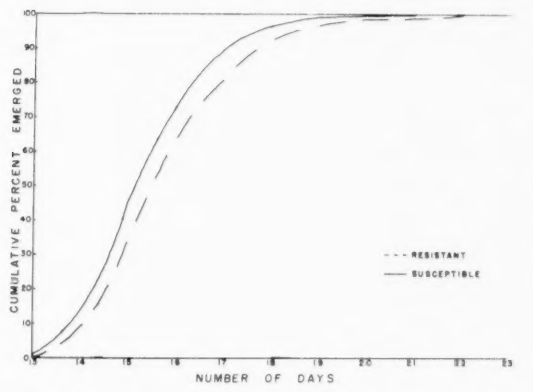

FIG. 2.—Length of developmental period of a resistant and susceptible strain of the boll weevil reared on a larval diet during the period July 15 to August 14, 1959.

Table 2.—Comparison of the weight of resistant and susceptible males and females.

Strain	Males		Females		L.S.D. at 5% Level
	No. Individuals	Mean Weight (mg.)	No. Individuals	Mean Weight (mg.)	
Resistant	1,205	11.23	1,118	12.40	0.05
Susceptible	1,225	11.19	1,134	12.51	.06

Table 3.—Comparison of the average reproductive potential of resistant and susceptible females.

Strain	No. Individuals	No. of Eggs per Female (avg.)	No. Eggs by One Female (min.)	No. Eggs by One Female (max.)
Resistant	134	114.98	2	678
Susceptible	136	146.68	2	574
L.S.D. at 5% level	31.62			

origin and were reared simultaneously under controlled conditions, it is concluded that these biological differences were linked with the differences of the strains in susceptibility to the chlorinated hydrocarbon insecticides.

Differences recorded of approximately one-half day longer developmental period and 22% reduction in fecundity in the resistant strain appear to be relatively slight. These differences were obtained under controlled laboratory conditions and could cause great differences in biotic potential of the boll weevil population exposed to the natural environment.

These results offer a possible explanation of why the susceptible boll weevil population predominated prior to the introduction of insecticides as selecting agents. However, the longer life cycle and lowered fecundity observed in the resistant strain of this boll weevil population may not occur in other strains of this insect as indicated by results of other workers cited in this paper.

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Combination Acaricide-Insecticide-Fungicide Sprays on Outdoor Roses[1]

T. J. HENNEBERRY, E. A. TAYLOR, F. F. SMITH, A. L. BOSWELL, and R. V. TRAVIS[2]
Entomology Research Division, Agric. Res. Serv., U.S.D.A.

ABSTRACT

Effective control of two-spotted spider mites, *Tetranychus telarius* (L.), on roses was obtained with weekly applications of Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite). Demeton and phorate applied every other week were effective early in the season but failed to give satisfactory control late in the season. Malathion did not give satisfactory control.

The highest yields in numbers of flowers cut and weights of flowers were obtained from plots having the most effective mite control.

Excellent control of blackspot was obtained with the fungicides maneb, zineb, ferbam, and captan. Maneb was the most effective, with zineb, ferbam, and captan decreasing in the order given.

Multipurpose spray or dust combinations for the control of pests enable the rose grower to maintain healthy, vigorous plants. The availability of such combination sprays or dusts is particularly desirable since insects, mites, and diseases of roses such as blackspot are often simultaneous problems in the growing season. Some combinations have been developed which are effective and the protection of rose plants from spider mite and blackspot injury has resulted in increased flower yields (McClellan *et al.* 1955, Taylor *et al.* 1956, Henneberry *et al.* 1960).

In 1957 an experiment was conducted at Beltsville, Md., to evaluate several new acaricide-insecticide-fungicide sprays for the control of rose pests. The factorial arrangement of treatment combinations also provided for the determination of compatibility and synergistic effects of various fungicides and acaricides.

METHODS AND MATERIALS.—Dormant roses were planted in April and set $2\frac{1}{2}$ feet apart each way in a plot with 5 feet between plots. Each plot consisted of 6 rose plants, 3 each of the varieties Helen Traubel and Red Radiance. The plants were mulched with 1 to 2 inches of sawdust. Applications of ammonium nitrate and 5-10-5 fertilizer were made at intervals and the roses maintained in good growing condition by periodic irrigations throughout the season.

The experiment was conducted in a randomized-block design consisting of 25 treatment combinations in 4 replications. The factorial arrangement (all combinations) of 5 acaricide and 5 fungicide levels was as follows: Acaricides—none, Aramite (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite), malathion, demeton, and phorate; and Fungicides—none, ferbam, maneb, zineb, and captan. All sprays contained 1 pound of DDT, 0.5 pound of lindane, and $\frac{1}{2}$ pint of Santomerse-S per 100 gallons.

The following tabulation gives the pounds of active ingredient per 100 gallons of water for each of the acaricides or fungicides:

Wettable powders:	
Aramite	0.30
Malathion	0.50
Ferbam	1.52
Zineb	1.30
Maneb	1.40
Captan	1.00
Emulsifiable concentrates:	
Demeton	0.83
Phorate	0.83

[1] Accepted for publication November 4, 1960.
[2] Formerly of Crops Research Division, Agric. Res. Serv., U.S.D.A.

The plots were sprayed weekly from June 18 to September 17 with the exception of demeton and phorate which were applied every other week. All sprays were applied with 2-gallon compressed-air sprayers under 60 pounds pressure.

Two-spotted spider mites, *Tetranychus telarius* (L.), were transferred from rose plants in older experimental plots to the Red Radiance variety and allowed to become established several weeks before treatments were begun. A spore suspension of blackspot fungus, *Diplocarpon rosae* Wolf, was sprayed on the Helen Traubel variety in the latter part of June.

The effect of treatments on mite populations was observed by counting the total number of living mites on 5 leaf-discs, $\frac{9}{16}$ inch in diameter, taken at random from the Red Radiance plant of each plot.

Blackspot ratings were made of the Helen Traubel variety on August 27 and October 4. Plants were rated on the basis of the percentage of foliage having blackspot lesions.

Yield records consisting of weights and numbers of flowers were taken twice a week in August and September. The flowers were cut just above the first 5-leaflet leaf.

Table 1.—Number of two-spotted spider mites per sample from Red Radiance rose plots receiving different acaricide-fungicide combination sprays.

Treatment Combination	Date Sampled				
	July 30	Aug. 16	Aug. 27	Sept. 12	Sept. 25
Check	183	242	321	187	122
Ferbam	69	144	80	106	90
Maneb	224	281	168	133	138
Zineb	80	158	140	143	109
Captan	68	41	152	154	100
Mean	125	173	172	144	112
Aramite					
Alone	3	92	34	2	0
Plus Ferbam	86	127	76	89	6
Maneb	21	111	36	30	7
Zineb	85	105	153	185	14
Captan	10	36	9	67	29
Mean	41	94	62	75	11
Malathion					
Alone	90	128	154	249	164
Plus Ferbam	134	136	96	91	69
Maneb	44	280	166	92	114
Zineb	128	147	58	181	92
Captan	173	49	47	219	226
Mean	114	148	104	166	133
Demeton					
Alone	10	43	205	186	132
Plus Ferbam	3	43	166	99	133
Maneb	12	82	128	243	115
Zineb	5	45	51	357	132
Captan	32	20	24	60	50
Mean	11	46	123	189	112
Phorate					
Alone	120	213	171	74	111
Plus Ferbam	27	128	43	103	84
Maneb	74	220	157	274	34
Zineb	48	63	68	238	126
Captan	33	190	187	226	69
Mean	54	163	125	182	85
L.S.D. at 5% level					
To compare means	12	78	69	60	44
treatment combinations	139	174	140	134	97

Table 2.—Mean and total yield per plot of Red Radiance roses receiving different acaricide-fungicide combination sprays.

Treatment Combination	August		September		Total Yield	
	Weight of Flowers (grams)	Number of Flowers	Weight of Flowers (grams)	Number of Flowers	Weight of Flowers (grams)	Number of Flowers
Check	515	66	405	51	920	117
Ferbam	583	64	780	70	1,363	134
Maneb	619	80	891	82	1,510	161
Zineb	557	58	533	54	1,087	112
Captan	715	75	854	82	1,569	157
Mean	598	69	693	68	1,291	136
Aramite						
Alone	793	89	1,041	100	1,834	189
Plus Ferbam	663	73	1,012	83	1,675	149
Maneb	706	84	836	77	1,567	161
Zineb	519	64	529	48	1,048	111
Captan	660	78	912	80	1,572	158
Mean	668	77	866	78	1,539	154
Malathion						
Alone	499	58	471	54	970	112
Plus Ferbam	533	59	734	69	1,267	128
Maneb	630	69	638	59	1,268	128
Zineb	451	67	555	51	1,006	104
Captan	604	64	851	69	1,455	128
Mean	543	61	650	60	1,193	120
Demeton						
Alone	808	80	729	79	1,536	159
Plus Ferbam	683	78	711	68	1,394	146
Maneb	750	99	719	74	1,539	173
Zineb	560	80	629	76	1,189	144
Captan	830	90	775	78	1,605	168
Mean	726	83	727	75	1,453	158
Phorate						
Alone	631	79	618	63	1,248	142
Plus Ferbam	683	75	807	85	1,497	160
Maneb	750	73	888	83	1,506	156
Zineb	716	80	729	71	1,445	152
Captan	663	69	756	83	1,419	152
Mean	664	75	760	77	1,423	152
L.S.D. at 5% level						
To compare means	121	14	N.S.	N.S.	245	22
treatment combinations	271	31	N.S.	N.S.	547	50

RESULTS.—*Acaricides.*—The effects of acaricide treatments on mite populations are shown in table 1. Weekly applications of Aramite reduced populations over the untreated check on each sampling date. Aramite with or without maneb or captan appeared to be the most effective. There were no significant differences between Aramite sprays with the exception of the Aramite-zineb combination on one sampling date (September 12). Malathion alone or in combination with any fungicide tested did not reduce mite populations compared with the untreated check. Demeton and phorate sprays applied every other week alone or in combination with any of the fungicides tested reduced mite populations early in the season. Phorate was not effective after July. Demeton-fungicide sprays were effective to the middle of August but gave inadequate control after that time.

Flower Yield.—The effects of acaricide-fungicide sprays on the yields of flowers from rose plants are shown in table 2. With the exception of malathion-treated plots which were not effectively protected from spider mite injury, the average weights of flowers from acaricide-treated plots were greater than those from untreated plots in August. When yields over the entire season were considered, higher weights of flowers were evident from Aramite-treated plots and more flowers were evident from demeton-treated plots in August and when the total yields for the season were compared. The yields from plots sprayed with Aramite-zineb combination were markedly reduced compared with plots sprayed with

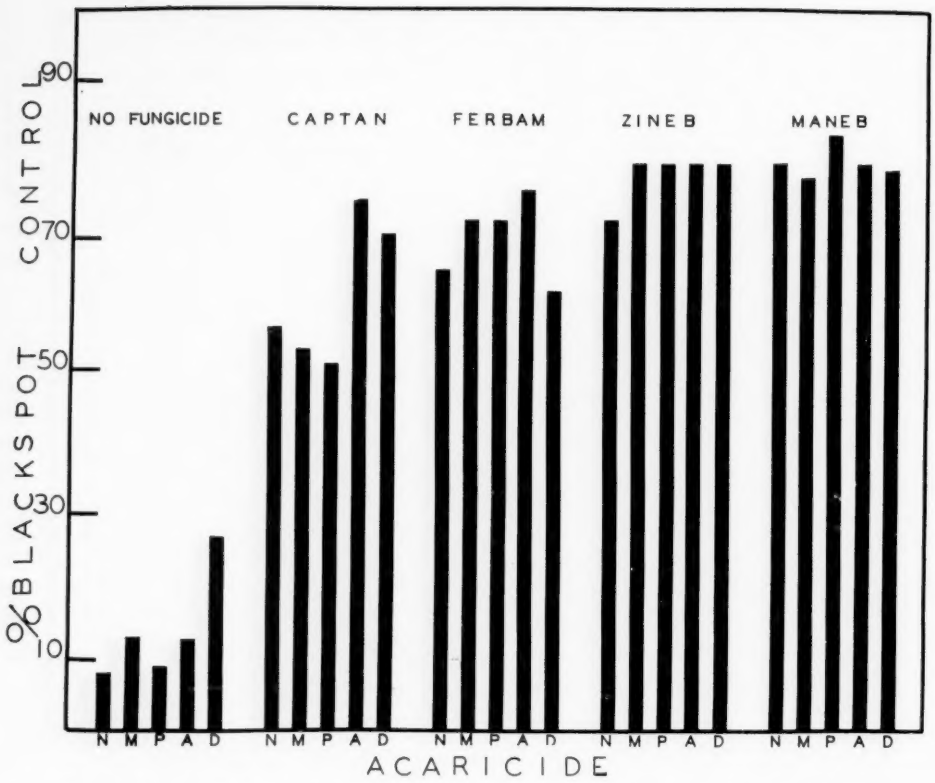

FIG. 1.—Per cent blackspot control on Helen Traubel roses with fungicides in combination with an acaricide: N, None; M with Malathion; P with Phorate; A with Aramite; D with Demeton.

Aramite or other combinations of Aramite-fungicide sprays. The differences, however, were not significant and no difference in plant growth was noted.

The yields from plants sprayed with the demeton-fungicide combinations were highest throughout the season, which can be attributed to the protection from spider mite injury afforded early in the season.

Fungicides.—All fungicides tested gave adequate protection from rose blackspot (fig. 1). Zineb or maneb were more effective than ferbam or captan. Yields of flowers were not significantly affected by fungicide treatments. This was caused apparently by the late blackspot infection. Subsequent injury to rose plants was not reflected in August and September flower yields.

There were no significant interactions. The acaricide and fungicide combinations were compatible and no plant injury was observed. The depression of mite control with Aramite and zineb or ferbam combinations and the reduced yields from plots sprayed with the Aramite-zineb combination may bear further investigation since no explanation is offered at this time.

The mite populations on fungicide check plots appeared associated with the extent of blackspot injury to the plant. The plant exhibiting the least defoliation and chlorosis often supported higher mite populations.

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Chemical Control of the Ground Pearl, *Eumargarodes laingi*[1]

WILLIAM T. SPINK[2] and JAMES R. DOGGER,[3] *North Carolina State College, Raleigh*

ABSTRACT

Controlling ground pearls, *Eumargarodes laingi* Jak. (Margarodidae: Homoptera), by using a nematocide and phosphate insecticides was studied in North Carolina during 1956–57. The nematocide, VC-13® (*O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothioate), gave some indications of control. Malathion plots showed the greatest reduction in posttreatment populations. Demeton was second in effectiveness. Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) gave no indication of control and posttreatment populations approximated those in the untreated check. A greenhouse experiment using the same phosphate chemicals and known pretreatment populations corroborated the field data.

Table 1.—Effect of treatment with VC-13 on populations of *Eumargarodes laingi* in tests at Wilmington, North Carolina, 1956.

	E. laingi IN FIVE 1-INCH SOIL CORES TRANSFORMED TO $\sqrt{x+0.25}$					
	Cysts (nymphs)			Adults		
PLOT	Pre-treatment	Post-treatment	Decrease (%)	Pre-treatment	Post-treatment	Decrease (%)
1	14.9	13.2	11.9	7.8	4.9	37.2
2	8.0	4.0	49.3	2.7	2.1	22.3
Check	6.8	7.4	(9.3)[a]	3.8	5.9	(55.6)[a]

[a] (Parentheses indicate increases.)

The ground pearl, *Eumargarodes laingi* Jak. (Margarodidae: Homoptera), was first discovered in Australia where it was found associated with the roots of sugarcane (Jakubski 1950). It has recently been found in lawns in the southeastern United States attacking the roots of St. Augustine grass (*Stenotaphrum secundatum*), centipede grass (*Eremochloa ophiuroides*), and bermuda grass (*Cynodon dactylon*) (Kerr 1957). The apparent injury to the roots results in brownish patches appearing in lawns seeded to these grasses. Extent of damage depends on the number of insects present. As many as 4,000 ground pearls have been found in a pint of soil (Kerr 1957). Infestations which developed in lawns at Wilmington, North Carolina, in 1956, caused concern which stimulated the initiation of experiments for controlling this pest with chemicals.

FIELD EXPERIMENTS.—*1956.*—A nematocide, VC-13® (*O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothioate),[4] was used in 1956. This material had shown promise as an insecticidal soil drench in previous tests on other insects. Two 10- × 10-foot plots, located on a centipede-grass lawn were treated June 1 with VC-13 at the rate of 8 ounces of 75% emulsifiable concentrate per 100 square feet. The material was added to 4 gallons of water and applied from a sprinkling can to each of the two plots. It was immediately washed into the soil with additional water from a sprinkling system which distributed water evenly over treated and untreated areas. Lawn adjacent to the two plots was used as a check.

Five pretreatment soil cores, 1 inch in diameter by 7 inches deep, taken with a soil auger were combined from each plot for each treatment into one sample. A similar sample was obtained from the adjacent untreated lawn used as the check. On June 8, 1956, posttreatment samples were taken in a like manner. At the time of treatment, adult ground pearls were found in the soil but had not deposited eggs.

The percentage decrease in population (table 1) between pretreatment and posttreatment counts (transformed to $\sqrt{x+0.25}$, where x=pretreatment counts) indicated some difference between treatments; however, adults in both treated and untreated plots appeared active and healthy. Two egg masses were found in the posttreatment sample of one treated plot and two were found in the posttreatment sample from the untreated plot. By the spring of 1957, the two treated areas were covered with an excellent stand of grass whereas the untreated area was in very poor condition.

1957.—A 9- × 12-foot infested area in another centipede-grass lawn was divided into 3- × 3-foot plots, in 3 blocks of 4 plots each. Treatments were randomized within each block. Pretreatment samples were taken on April 26, 1957, in lots of five 1-inch core samples, 7 inches deep. Treatments were made with wettable powder Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) in water suspension, and demeton and malathion as emulsifiable concentrates. These were applied as soil drenches. Each plot was treated separately and was surrounded by four 3-foot shields 18 inches high made of galvanized sheet metal. Each shield had two strap-iron stakes welded to the back, which extended 5 inches below the shield. The shields were driven approximately 1 inch into the soil to avoid seepage of insecticides into adjoining plots. Materials were applied evenly over each plot with a watering can and then washed into the soil with water from a hose. Initial applications were made when first-instar nymphs were first found in the soil June 19, 1957. A second application was made July 2.

The most vulnerable stage of the insect to insecticides was thought to be the first nymphal instar, an unprotected, migratory and feeding stage; thus insecticides were applied when this stage predominated. Subsequently, adults were reared from cysts taken from treated plots, which would indicate some protection from insecticides is provided by the cyst wall.

Posttreatment samples were taken October 23, 1957. During the lapse of time between pretreatment and posttreatment counts, the first-instar nymphs secreted wax cysts, which made it easier to isolate them from the soil. A paired sampling technique was used in which the post-

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[2] Now Assistant Professor, Entomology Research Department, Louisiana State University, Baton Rouge.

[3] Now Head, Entomology Department, North Dakota Agricultural College, Fargo.

[4] Product of the Virginia Carolina Chemical Corporation, Richmond, Virginia.

Table 2.—Posttreatment population of *Eumargarodes laingi* in field tests at Wilmington, North Carolina, 1957.

Treatment	Active Material per Acre (lb.)	Posttreatment Means[a] Transformed to $\sqrt{x+0.25}$		
		Means Unadjusted	Means Adjusted[b]	Means Decoded
Demeton	1	6.07	5.10	25.8
Check	0	4.66	4.83	23.1
Dylox	2	3.74	4.45	19.6
Malathion	4	2.97	3.06	9.1

[a] Insects per 1-inch soil core.
[b] Adjusted to an average pretreatment count of 4.94.
L.S.D.—1.53@ 5% level.
—1.83@ 1% level.

Table 3.—Mean populations of *Eumargarodes laingi* in pots in greenhouse tests at Raleigh, North Carolina, 1957.

Treatments	Actual Mean Population	Transformed to $\sqrt{x+0.25}$	Decoded
Check	43.3	6.13	37.3
Dylox	22.4	3.79	14.1
Demeton	10.5	1.80	3.0
Malathion	4.8	1.68	2.6

L.S.D.—1.84@ 5% level.
—2.46@ 1% level.

treatment sample was taken within 2 to 3 inches of the randomly selected pretreatment sample. This was done to reduce variability between sample counts as much as possible.

An analysis of covariance[5] wherein the posttreatment counts were adjusted for pretreatment counts indicated little difference among individual regression coefficients. Therefore, assuming a constant regression coefficient, the differences between adjusted posttreatment means were tested when each mean was adjusted to a common pretreatment count of 4.94 on the transformed scale.

The data in table 2 show that only malathion-treated plots had significantly lower posttreatment populations of ground pearls than the untreated checks.

GREENHOUSE EXPERIMENT.—*1957.*—The following study was made to test the effectiveness of 3 phosphate insecticides on a known population. Black sandy soil was brought from Wilmington, North Carolina. This soil was obtained from areas in which it was reasonable to assume that cultivated grass had never grown. It was sifted through 4-, 17-, and 30-mesh screens which eliminated all foreign materials leaving only a silt-like sandy loam. This soil was then autoclaved at a temperature of 240° F. for 90 minutes at 15 pounds of pressure. Six-inch diameter flower pots were sterilized at the same time. Sod of centipede grass was obtained from a Raleigh, North Carolina lawn which was free of cysts. The soil was carefully removed, the roots were washed under running tap water and small sprigs of grass were planted in each of the pots. All of the pots were buried to the lip in sterilized sand on concrete benches. Each bench had provision for drainage at the bottom. Water was furnished to the grass indirectly by watering the surrounding sand until surplus water drained through the opening at the bottom of the bench. This procedure was followed as previous tests in pots had shown that too much water in the soil killed the first instar nymphs. The same phosphate chemicals were used at the same rate of concentration used in the field tests of June 19, 1957 (table 2). An untreated check was included. Each replicated block consisted of individually treated pots in randomized positions. Two benches provided space for 16 replications. Ten adult females were introduced into each pot to a depth of 1 inch. On June 18, 1957, the insecticides, suspended in 100 ml. of water, were applied to the pots as a drench. An additional 100 ml. of water was used to wash the insecticide into the soil. A small quantity of 6-8-6 fertilizer was added to all pots. Pots not receiving insecticide treatmerts were irrigated with 200 ml. of water. This was the only liquid added to the soil directly. Soil temperatures were taken in pots at both ends of each bench and the average temperature calculated for each bench. Soil temperatures in the greenhouse were adjusted to approximate summer temperatures at Wilmington, North Carolina, by using a canopy of dark muslin over the benches and by whitewashing the greenhouse glass panes. On November 15, 1957, the soil and roots of the grass in the pots were examined for the presence of ground pearls.

An analysis of variance of the ground pearl populations in the variously treated pots in the greenhouse test showed highly significant differences between treatments. The coefficient of variation of this experiment was 77%. Data shown in table 3 indicate that all treatments differ significantly from the check.

CONCLUSIONS.—Distribution of ground pearl populations in the soil has some bearing on sampling results and therefore affects data obtained on control in field experiments. The use of parallel tests, using controlled populations as was done in the greenhouse experiment, helped corroborate field data. Analysis of the data from both field and greenhouse experiments demonstrated that reductions of populations in treated field plots were due in part to the action of the insecticides. Malathion was the most effective of the materials tested.

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Flight Distances of Drosophila Determined with Radioactive Phosphorus[1]

A. P. Yerington[2] and R. M. Warner[3,4]

Abstract

The flight range of *Drosophila* spp. has become increasingly important to the vineyardist and fig grower of the San Joaquin Valley of California due to the increased intermixing of various other fruit and vegetable crops and their attendant cull disposal problem. Drosophila were tagged with P^{32} on fermenting fruit paste and released in the field. Several types of bait traps were used to recapture the insects. *D. melanogaster* Meig. was trapped at a distance of at least 4.4 miles upwind from the release point after 24 hours.

Species of *Drosophila*, particularly *D. melanogaster* Meig., cause serious economic losses to growers of figs, grapes, tomatoes, and some other crops in California by introducing spoilage organisms and by their undesirable presence in the fruit (Warner 1958). In recent years control of vinegar flies on figs and grapes in the central San Joaquin Valley of California has become increasingly difficult because of the increase in other crops grown in proximity and providing organic residues which are breeding places for drosophila. A succession of crops, starting in early June with apricots and plums and continuing later with peaches, watermelons, cantaloups, tomatoes, and finally grapes and figs extending into October, furnishes continuous series of breeding places. Because there is a gradual buildup of vinegar flies throughout the season, heavy concentrations of these insects are found in the latest of the harvested crops, figs and grapes (Yerington 1959). Chemical control in fig orchards, as now developed, is effective for only 2 or 3 days because of immediate reinfestation from outside sources and rapid breakdown of the insecticides. It thus becomes apparent that problems of flight distances and directions are of primary importance in the development of control measures for this vinegar fly. In the experiment reported in this paper, a total of eight tests were conducted over a two-year period to determine distance of flight. Because of the results obtained in the eighth test, data pertaining to the test are included in the discussion at the end of the paper.

Insects.—*Drosophila melanogaster* was used in the tests; however, when the test insects were field-collected it proved to be impractical to exclude the few other species present (analyses of field populations showed that more than 95% of the drosophila collected during the summer and fall were *D. melanogaster*). The flies used in tests 1 and 2 were laboratory-reared flies from both the Stored-Product Insects Laboratory and the California Fig Institute Research Laboratory. The cultures were started from wild specimens captured in the area where the tests were conducted. In tests 3 and 4 the flies were tagged in the field near the Chowchilla, Madera County, city dump. The fifth release was made with flies netted in a fruit dump near Clovis, Fresno County. In the 1959 releases, tests 6 and 7, the flies were netted in the stem piles from a winery. The populations were so heavy in these areas at times that up to 70,000 flies would be netted in less than 20 minutes.

Tagging.—Various experiments with dyes as markers (Dalmat 1950) were attempted with poor results. Pimentel & Fay (1955) had used P^{32} successfully in tagging drosophila, so this method was adopted. For tagging in the laboratory the flies were held without food a few hours or overnight, inactivated in the refrigerator at about 38° F., and poured into 1-gallon glass jars. At the bottom of each jar was a petri dish or a paper freezer carton containing about 3 ounces of fermenting ground fig bait. An aqueous solution of P^{32} was distributed over the surface of the bait with a 1-ml. hypodermic syringe. At least 1 ml. of liquid was found to be necessary to give good distribution over the surface. The P^{32} was diluted so that 250 to 300 microcuries were contained in 1 ml. of liquid. Flies fed on this bait picked up 200 to 1,100 or more counts per minute in a period of 18 to 20 hours. The females picked up heavier counts than the males.

For tagging drosophila, in the field, the bottom one-third of a gallon glass jar was filled with fermenting fig bait. Three layers of muslin and a ring of cardboard, cut so it would hold down the edges of the cloth, were placed on top of the bait. This arrangement prevented flies from entering the bait but allowed the odors to escape. A paper carton or petri dish containing a small amount of radioactive bait was placed on the cloth cover. Thus a large amount of bait emitted attractant odors but only a small quantity of bait was actually tagged, and the problem of disposing of the radioactive waste was reduced. Cone-trap tops on the jars retained the flies so they would take up more P^{32}, give a stronger count, and be easier to detect upon recovery. The jars were placed at ground level inside a sturdy weather-instrument shelter, located in the shade and padlocked so no animal or passerby could get into it. The traps and baits were removed after 2 days so the tagged flies could leave the shelter.

During the hot portion of the season in the San Joaquin Valley, laboratory-tagged flies were carried to the field in a portable refrigerator. Temperatures around 100° F., which were common in the afternoon, were lethal to confined drosophila, especially in their state of high excitement resulting from transportation. Stafford (1959) reported 100% kill of closely confined *D. melanogaster* in 80 minutes at 100°.

For counting the tagged flies and checking the P^{32}, a transistorized beta-gamma survey meter with an end window tube was used (Universal Atomic Geiger Counter No. 405[5] modified by the Radiation Safety Division of the University of California by inserting an Anton No. 201 Geiger-Muller Tube). The counts per minute were indicated on a dial or could be detected with an earphone.

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[2] Entomologist, Stored-Product Insects Laboratory, Agricultural Marketing Service, U. S. Department of Agriculture, Fresno, California.

[3] Research Director, California Fig Institute, Fresno, California.

[4] The authors gratefully acknowledge the counsel and technical assistance of Dr. John M. Heslep, Chief Radiological Safety Engineer, and C. E. Smedley, Radiation Safety Division, University of California, Berkeley, California.

[5] The use of trade names in this paper is for identification purposes only and does not constitute an endorsement of the product by the U. S. Department of Agriculture.

TRAPS AND BAITS.—Several types of traps were used in recapturing the tagged flies. A plastic cylinder trap designed by Steiner (1957) was modified at the Stored-Product Insects Laboratory at Fresno by inserting a cone of 30-mesh copper screen into each of the two end openings. The entrance holes were reduced to a diameter of one-quarter inch so that larger species of flies could not enter. A cotton dental roll about 2 inches long, treated with several drops of DDVP, was fitted inside the trap. The bait consisted of a 1½-inch piece of banana covered with about 1½ ounces of a yeast suspension in a 3¼-ounce paper cup. One ⅔-ounce packet of Fleischmann's granular, active, dry yeast was dissolved in 20 fluid ounces of distilled water to make the suspension. Counting was facilitated by the fact that the flies were dead and dry except for a few that fell into the liquid.

A second type of trap, simple and effective, was developed by members of the research staff of the California Fig Institute. A cone made of organdy (diameter 3 inches) was attached to the screw top of a wide-mouth pint jar by gluing it to the flanged rim of the screw-on portion of the lid. The cone extended down into the jar about 1¼ inches. The hole in the bottom of the cone was about one-eighth inch in diameter. Organdy was used because it was firm and allowed a good volume of the bait odors to escape.

The bait was made from cull dried figs soaked with an equal volume of water until soft and then ground into paste. The paste, of fairly moist consistency, was inoculated with Fleischmann's active dry yeast at the rate of one ⅔-ounce packet to each 2 or 3 gallons of figs, mixed well, and allowed to ferment overnight. About 3½ ounces of this bait was placed in each jar. One-quarter teaspoon of 25% Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) or premium-grade malathion wettable powder was sprinkled over the bait and allowed to remain on the surface where insects could contact it. Any repellency of the insecticide apparently was overpowered by the volume of attractive odors emitted by the bait.

It was difficult to remove the flies from the surface of this wet bait for counting. To avoid this Stafford & Kido (personal communication) developed a basket to fit inside the trap between the cone and the bait (fig. 1). The basket was made as follows: Two lengths of 2-inch butchers' tape, each equal to the circumference of the inside of the trap, were formed into two circular bands. One band was placed inside the other with the glued sides together. The bottom of the basket was a circular piece of organdy inserted between the two bands and held in place by gluing the bands together. The upper edge of the basket was slit in several places and bent out to hold the basket in the neck of the jar. Insecticide placed on the organdy killed the flies so that collecting and counting them was simplified. For this purpose a solution of 50% Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) soluble powder proved satisfactory.

FIG. 1.—Improved model of gallon-jar trap: (Right), screw top of jar and organdy cone; (Center), jar with bait; (Left), basket.

The basket described above was adapted to both gallon- and pint-jar traps and was used in all the 1959 traps. The gallon jars held a much greater volume of attractant and therefore fewer of them were needed. Whole figs were used in the bait in a formula described by Warner (1958) for orchard use by growers.

FIELD PROCEDURES.—To determine flight distances, releases of tagged flies were made in open areas where there were not many orchards or fruit dumps to divert the flies. Michelbacher & Middlekauff (1954) reported that drosophila move chiefly upwind; therefore, the release points were located downwind from isolated fig orchards.

Before a release, traps of various types with fermenting bait were widely spaced in shaded or otherwise protected sites. The radioactive bait was removed from the jars in the laboratory before the flies were taken to the field. At the release point the jars were opened and many of the flies left immediately. The remainder were scattered onto a 3″×4″ piece of white paper. Only a few of the flies remained after 15 or 20 minutes.

The flies were collected from the traps, in vials, at 24- to 48-hour intervals and taken to the laboratory for identification. Where the flies were on the bait surface (pint-jar traps), the trap with its entire catch was taken in at the end of the experiment and checked as a whole for radioactivity because of the difficulty of isolating single tagged flies. In some instances the flies were removed and counted to compare total numbers of flies with total numbers of tagged flies in different traps. For the other types of traps the dead flies were poured onto a sheet of paper and the counter run over them. Tagged flies could be detected and counted easily by this method. When large numbers of flies were trapped the numbers were estimated by measuring them in calibrated vials.

INDIVIDUAL TESTS.—Many of the details regarding the releases are given in table 1.

Test 1. This release, on August 7, 1958, was made at 10:00 p.m. to avoid the high afternoon temperature. At 7:00 p.m. the temperature was still 90°. Fallow ground and some pasture land surrounded the release point. At least two tagged flies flew over several traps in order to reach the Calimyrna fig orchard 1 mile to the north.

Test 2. This release, 5 days after the first, was made in Dry Creek reservoir 4 miles northeast of Clovis. One tagged fly was recovered in the fig orchard 1 mile northwest of the release point. A 35-foot earthen dam was between the release point and the orchard.

Tests 3 and 4. These flies were tagged in Berenda Slough, 25 yards from the Chowchilla city dump. A large

Table 1.—Description and results of seven releases of tagged drosophila to determine flight distances.

Release Number	Estimated Number of Flies Released	Date of Release	Traps			Weather 7 p.m.[b]			Trapping Record			Flight Record		
			Number	Type	Location[a]	Temperature (° F.)	Wind Direction	Wind M.p.h.	Hours after Release	Flies Caught	Tagged Flies	Tagged Flies	Miles Distant	Direction
		1958												
1	2,000[c]	Aug. 7	13	St[d]	5 orchard	90	NW	10	12	42	8	2	1.0	N
					6 flight path				36	29	1	7	0.2	S
					2 S[a]									
2	4,000[c]	Aug. 12	12	St	8 orchard	92	WNW	6	17	53	1	1	1.0	NW
					2 flight path				40	83	1	1	0.5	SE
					2 SE									
3	Tagged in field	Sept. 26–28	8	St	Orchards	77	W, calm	4	72	15,611	1	1	1.5	NW
			24	pint	1.3–3 mi.	to	and							
					N & NW	80	WSW							
4	Tagged in field	Oct. 3–5	8	St	Orchards	79	WSW	7	72	—	1	1	1.5	NW
			24	pint	1.3–3 mi.		NW							
					N & NW									
5	20,000[c]	Oct. 25	21	St	38 orchard	64	SSW	6	48	40,179[f]	127[f]	36[f]	2.4	N
			24	pint	3 flight path		NE					34[f]	2 –2.3	N
					4 S							57	0.1–2	N & NW
		1959												
6	60,000[c]	Sept. 16	28	St	39 orchard	65	NW	10	24	29,736	5	6	3.4	N
			20	gal.	17 S, E, W	to	E		120	—	5	2	3 –3.3	N
			8	pint		71						1	1.2	E
												1	1.0	SE
7	40,000[c]	Oct. 13	8	St	31 orchard	76	NW	6	24	148,300	61	7	4.4	N
			20	gal.	7, S, E, W				48	245,390	44	6	4.0	N
			10	pint					72	—	9	53	1.5	E
												48	0.2–1.2	S

[a] Direction from release point.
[b] U. S. Weather Bureau, Fresno Air Terminal, (5 miles east of Fresno and 3 miles south of Clovis).
[c] Laboratory reared and tagged.
[d] Steiner trap.
[e] Field-collected, laboratory-tagged.
[f] Steiner trap counts only.

number of flies were present on about 97 truckloads of cull fresh tomatoes dumped there over a period of several weeks. Two 1-gallon traps caught 20,000 to 25,000 flies overnight. The experiment was conducted to determine if these drosophila moved from the dump into Mission fig orchards, which were 1.3 miles to the northwest. One tagged fly was recovered in the fig orchards 1.5 miles to the northwest from each of these releases, an indication that some of the flies moved out of the dump even though large quantities of attractive food were present. During this test it was found that the flies did not acquire enough radioactivity when allowed free access to the bait, *i.e.*, in open-top containers. Therefore, cone-top traps were applied to the bait jars and flies were held for a period of 24 to 48 hours before they were released in subsequent tests.

Test 5. For this test on October 25, 1958, 20,000 flies netted at a fruit dump were tagged and released 9 miles east of Clovis. The release point was 2 miles south of an isolated Calimyrna fig orchard. Traps were placed at intervals on the outer rows of fig trees around the entire orchard and a few traps were placed on the line of flight between the release point and the orchard. A total of 127 tagged flies was captured in the Steiner traps, 64 of which were trapped in the fig orchard. Of the 64 tagged flies trapped in the orchard 26 were caught in the extreme northern row of the orchard which was 0.4 mile farther north than the southernmost row. It is believed that the flies, attracted to the orchard and the baits, moved through the orchard to the north edge and stopped there because only dry grasslands were beyond. No traps were located beyond this point. Only catches in three of the Fig Institute traps were counted but they totaled 14,110 flies, including 20 tagged ones. However, catches in 18 of the 24 Fig Institute traps used in this test, both in the orchard and along the flight path, showed radioactivity indicating the presence of tagged flies.

Test 6. This release, made on September 16, 1959, was at the same location as test 5, the only difference being that the release point was 3 miles south of the fig orchard. A storm occurred just after the release of the flies and 0.89″ of rain was recorded on September 18. Six tagged flies were recovered at the north edge of the orchard, having covered a total distance of 3.4 miles.

Test 7. The same site was chosen for this test as for tests 5 and 6. Release was made on October 13, 1959, 4 miles south of the orchard, at 5:00 p.m., under ideal conditions (temperature about 80° F. and wind 0.3 m.p.h.). Twenty-four hours after release, 148,300 flies, including 61 tagged drosophila, were collected from the traps. Forty-eight hours after the release time, an additional 245,390 flies were captured, among which were 44 tagged individuals, some of which had flown 4.4 miles. At 72 hours after release nine additional tagged flies were collected. No attempt was made to count the total number of flies in this period. The counts in this test illustrate the value of the tagging method. Except with a radioactive tag, the task of detecting the few released flies among so many would be insurmountable in terms of time and effort.

Discussion.—An eighth test, which is not listed in the table, was run in Madera County. A release of 20,000 tagged flies yielded a complete blank so far as recovery in

the traps was concerned and only a small number of untagged flies was captured apparently because of the steady 12- to 14-mile wind which blew during a 48-hour period. Although the flies appear to move into the direction of the light prevailing winds up to 10 m.p.h., they do not fly at all when there is a higher wind velocity. The prevailing wind direction in the San Joaquin Valley is from the northwest, although along the foothills east of Clovis gentle southeast winds are frequent in the morning until about 10:00 a.m. Temperature and wind are the most critical factors influencing the flight of drosophila. Conditions are most frequently favorable for flight in the hours just before sunset; therefore, the 7:00 p.m. conditions are reported (table 1) as indications of the optimum daily weather.

In tests 5, 6, and 7 many of the tagged flies caught were in the most distant traps. We believe that 4.4 miles is by no means the extreme range of drosophila.

During test number 7 a few dried-fruit beetles, *Carpophilus hemipterus* (L.), were accidentally captured and tagged along with the drosophila. Two of these beetles were recaptured at the extreme range of the traps, 4.4 miles. This extends the previous known flight range of 2.5 miles reported by Simmons and others (1936).

It is not known how far vinegar flies can detect odors, but we believe that fruit orchards produce aromas which attract from a considerable distance. During high temperatures, drosophila remain in cool shady locations. They move when the population is high, there is little or no wind, low light intensity prevails, and temperatures are between 55° and 85° F.

Before this work was begun, it was generally thought that drosophila would move at most a few hundred yards. That they can fly at least 4.4 miles is a partial explanation (coupled with the rapid chemical breakdown of insecticides) for many failures of attempted chemical control on small acreages. Garbage dumps, fruit dumps, and unharvested crops produce vast numbers of drosophila. They are known carriers of spoilage diseases and their presence causes infestation of many fruits. It is evident that chemical control measures must be coordinated with the reduction or elimination of the breeding places within 4 or 5 miles of susceptible crops. This suggests the need for area control programs.

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Practical Application Methods for Systemic Cattle Grub Control[1]

EARLE S. RAUN and FRANK E. FRENCH[2]

ABSTRACT

The cattle grubs *Hypoderma lineatum* (de Vill.) and *H. bovis* (L.) were controlled through various methods of applying three systemic insecticides: Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), Dowco 109 (*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidothioate) and Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate).

In a series of tests these three systemic insecticides were applied at low pressures (80 to 100 pounds). Grub control by Co-Ral was excellent and unaffected by the addition of household detergent at the rate of one-half pound per 25 gallons of spray.

Backrubber applications of 5% Ruelene as an oil solution failed to systemically control the cattle grubs.

Spray-Dip machine applications of Co-Ral and Ruelene were moderately effective. No increase in gains in weight attributable to the systemic grub control was observed.

The use of systemic insecticides for the control of cattle grubs has been well demonstrated both experimentally and in the field. The problem now facing the livestock industry is to educate and persuade the livestock producer to universally utilize these systemics, thus eliminating the losses caused by the cattle grubs *Hypoderma lineatum* (de Vill.) and *H. bovis* (L.). Every possible method of application of systemic insecticides must be tried so that the most efficient and convenient ones will be recommended to the cattle producer. To this end the field tests reported in this paper were conducted.

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[2] Professor and graduate assistant, respectively, of the Department of Zoology and Entomology, Iowa State University of Science and Technology, Ames.

The tests of application methods reported here were carried out during several seasons. One series of tests utilized low pressure applications of systemic insecticides with and without detergent. A second test compared the Spray-Dip[3] machine with the more conventional orchard-type sprayer for the applications. Backrubbers applying small quantities of insecticide daily were utilized in another test.

LOW PRESSURE SPRAY APPLICATIONS.—*1957.*—Two different groups of cattle were utilized in tests of the efficacy of Co-Ral[4] (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) and Dowco 109 (*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidothioate) as low pressure sprays. Herd A was a cow-calf breeding herd of purebred Angus cattle. It was divided into 3 groups; 2 of them containing 14 calves and the third with 15 calves. The calves weighed approximately 300 pounds each at the time of treatment in mid-August. Each calf was ear-tagged so that they could be pastured together but still be identified at "grub-counting" periods.

One group of 14 calves was sprayed with 0.5% Co-Ral suspension at the rate of 2 quarts per head. Two quarts of 0.5% Dowco 109 suspension were applied to each of the calves in the 15-head group. The remaining 14 head were left untreated as controls.

Dreft[5] (a detergent of Procter and Gamble) was added to the suspension of each systemic at the rate of one-half pound per 25 gallons of spray material. The sprays were applied only to the topline of each animal while the group was confined in a small corral. The spraying pressure of 80 p.s.i. was supplied by a nylon roller pump powered from a tractor power-take-off shaft. A spray gun applying a solid cone pattern was used. The gun was held at varying distances from the calves since all spray was applied while the operator stood on the corral fence. To evaluate the control obtained through the use of the materials, grub counts were made on March 17 and April 23, using the palpation method, while the animals were confined in a chute. The results of the treatments indicated excellent grub control with Co-Ral as applied under these conditions (table 1).

Herd B, 22 Hereford calves, was treated on December 3. Dowco 109 was applied as a 0.65% suspension at the rate of 2 quarts to each of five calves. Co-Ral was used in the same way on eight calves as a 0.5% suspension. These calves averaged approximately 420 pounds at the time of treatment. Nine calves were untreated as controls. Grub counts were made on February 18 and March 31 using the palpation method. Treatments were applied with the same equipment, pressures and detergents utilized on Herd A. Results of these treatments again show good systemic activity with Co-Ral when it is applied with pressures not exceeding 80 p.s.i. (table 1).

1959.—In this field test low pressure applications were made comparing suspension sprays of Co-Ral with and without the addition of a detergent. Calves averaging 400 pounds in weight were put into the test on November 20. These were from the cow-calf herd owned by the Iowa State University Animal Husbandry Department and pastured the summer of 1959 on the Beef Nutrition Farm northwest of Ames. The 56 calves were allotted on a random basis to one of the three treatments.

Table 1.—Effect of low pressure sprays[a] of Co-Ral and Dowco 109 against cattle grubs.

CATEGORY	HERD A			HERD B		
	Co-Ral 0.5%	Dowco 109 0.5%	Untreated	Co-Ral 0.5%	Dowco 109 0.65%	Untreated
Number of calves	14	15	14	8	5	9
Calves grub free (%)	100	33	29	88	60	0
Grubs/calf (range)	0–0	0–8	0–8	0–7	0–26	4–29
Grubs/infested calf (avg.)	—	3.6	3.2	7	14	10.7
Control of grubs (%)	100	0	—	92	48	—

[a] Two quarts per calf.

Table 2.—Effect of detergent on low pressure applications of Co-Ral (3 quarts 0.5% per animal) for cattle grub control.

CATEGORY	DETERGENT[a] ADDED	WITHOUT DETERGENT	UNTREATED
Number of animals	19	19	18
Animals grub free (%)	95	100	39
Grubs/animal (range)	0–3	0–0	0–47
Grubs/infested animal (avg.)	3	—	11
Control of grubs (%)	99	100	—

[a] One-half pound of Tide per 25 gallons of spray.

Two of the groups of calves received an application of 0.5% Co-Ral as a suspension. Tide[6] (a detergent of Procter and Gamble) was added at the rate of one-half pound per 25 gallons to the Co-Ral suspension applied to one of the two groups. No detergent was added to the suspension applied to the other lot of calves.

The equipment used was a jeep-mounted power-take-off pump, hose and handgun. The sprayer was operated at 80 to 100 pounds pressure during applications. The treatments were applied to 10 or 12 calves at a time in a small holding corral, while the operator was standing on the corral fence. The major amount of material was therefore applied to the topline of the animals. The suspension was applied at the rate of 7.5 gallons to each 10 head of animals as nearly uniformly as possible, making an average of 3 quarts of suspension per calf. The third group of calves received no treatment and were kept as checks.

Grub counts were made to evaluate the treatments using the palpation method while animals were restrained in a chute. Counts were made February 3, March 1 and April 26. At the time of the first count only large, mature grubs were counted but when the other two counts were made, all grubs present in the backs of the animals were recorded.

The data obtained (table 2) indicate that there was no difference in the effectiveness of Co-Ral as a grub control systemic when applied either with or without a detergent at 80 to 100 pounds pressure. Both treated lots of calves were essentially free of grubs.

SPRAY-DIP MACHINE VS. ORCHARD SPRAYER, 1959.—Many of the cattle coming from range states to mid-western feedlots go through sale barns and commission firms. A large, speedy and effective machine placed in

[3] Livestock Sprayer Manufacturing Company, San Jose, California.

operation for the application of cattle grub systemics at these locations would make it very simple for feeder cattle to be treated. Such a machine is the Spray-Dip machine which consists of an all-metal, box-like chute, with solid metal doors. The Spray-Dip machine applies insecticide suspensions through 27 nozzles positioned to completely wet the animal inside the chute.

In order to determine the efficiency of the Spray-Dip machine, tests were carried out using a Hardie orchard sprayer for comparison in effectiveness of applications of the insecticides Co-Ral and Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate).

Ninty-seven head of Hereford calves were allotted to the treatments on October 27, 1959. These cattle had summered together on Kansas wheat pastures and had originated from the same Colorado ranch. They were gate-cut into four lots. A lot containing 26 head was designated as control and the cattle of the other three lots were each given one of the systemic treatments. Previous to this, they had been individually weighed on October 20 and fitted with neck-chain identifications.

The Spray-Dip machine was used to apply a suspension of 0.5% Co-Ral at the rate of 1.1 gallons per head to the cattle in one lot. The same machine applied the same gallonage of 0.5% Ruelene to the 24 head of the second lot. The third treatment consisted of an application of 0.5% Co-Ral at 1 gallon per head using the Hardie orchard sprayer which was operated at 150 to 250 pounds pressure.

On March 17, 1960, the test cattle were again weighed individually. They were slaughtered the next day. Grub counts were made on the carcasses and hides in the packing plant immediately after the hides were pulled from the animals (table 3). The data on weights were analyzed statistically and again the increase in weight gained by the treated animals was not significant (table 3).

BACKRUBBER TEST, 1959.—In late May, backrubbers applying toxaphene were installed in the pasture of a herd of cows and their April-born calves. The herd utilized these backrubbers during the summer and excellent control of horn flies (*Haematobia irritans* (L.)) was obtained. The backrubbers used in this test were of the suspended-tank type, with curtain-like wicks hanging beneath the tank.

On August 28, one-half of the herd was moved to an adjoining pasture and backrubber units applying toxaphene were moved also. In the original pasture the remaining backrubbers were filled with an oil solution containing 5% Ruelene. Cattle continued to utilize these backrubbers until October 27 when they were moved to winter quarters and the calves were separated from the cows.

Table 3.—Applications of 0.5% Co-Ral or Ruelene made with Spray-Dip machine or Hardie orchard sprayer at the rate of 1 gallon per animal for control of cattle grubs.

CATEGORY	SPRAY-DIP		HARDIE SPRAYER[a]	
	Co-Ral	Ruelene	Co-Ral	UNTREATED
Number of animals	23	24	24	26
Avg. gained (lb.)[b]	442	439	429	414
Animals grub free (%)	22	50	38	0
Animals with fewer than 5 grubs (%)	70	83	88	42
Grubs/animal (range)	0–13	0–8	0–9	1–15
Grubs/infested animal (avg.)	4.7	3.5	3.8	5.6
Grub control (%)	34	69	57	—

[a] Applied at 150 to 250 pounds pressure per square inch.
[b] Average total gain per animal in 150-day period.

Table 4.—Effect of backrubber and low pressure spray applications of Ruelene against cattle grubs.

CATEGORY	BACKRUBBER (5% IN OIL)	SPRAY, 0.5% (2 QUARTS PER ANIMAL)	UNTREATED
No. of animals	20	10	10
Grubs/animal (avg.)	10.9	0.1	5.6
Control (%)	0	98	—

Of the calves utilizing the toxaphene backrubber, one-half were sprayed on October 28 with 0.5% Ruelene applied at 80 pounds pressure. Approximately 2 quarts were used per head. A nylon roller pump on a tractor power-take-off shaft provided the pressure to a hose and orchard-type handgun. The other toxaphene-treated calves were not given a grub treatment but were kept as controls. Grub counts were made in all three groups of animals on February 15, 1960. The palpation method of counting showed that of the three groups, only the Ruelene-sprayed calves were essentially grub-free (table 4).

DISCUSSION.—Many Midwestern stockmen have access to low pressure sprayers that will develop approximately 100 p.s.i. The tests reported in this paper indicated conclusively that effective grub control may be obtained with systemic insecticides applied as low pressure sprays. Detergents added to facilitate "wetting" by the suspensions, while perhaps not necessary when sprays were carefully applied, do not detract from the effectiveness of the material.

Two to three quarts of a suspension applied per calf (weighing 250 to 600 pounds) is sufficient quantity even though the calves are long-haired in preparation for winter. In previous tests the authors have observed rather large amounts of "run-off" when greater amounts were applied to calves of similar size.

In these tests the calves that received spray treatments were crowded into small holding pens or chutes and were sprayed on the topline (back region). Thus the sprayer operator could quickly and easily apply the designated treatment. Undoubtedly, however, if the treatment is for an infestation of lice a rather determined effort should be made to treat the animal "all over."

In the low-pressure tests, Co-Ral gave from 92% to 100% control when grub counts obtained from the treated animals were compared with those taken on untreated calves. Dowco 109 gave from no control to 48% control with the concentrations applied.

In a comparison of the effectiveness of applications made with the Spray-Dip machine or the Hardie orchard sprayer, the control by both Co-Ral and Ruelene was less than expected. Further tests with the Spray-Dip machine may demonstrate effective control of cattle grubs by this method. All three lots that were treated with a systemic insecticide showed an increase in weight

gained over the untreated lot (table 3) but it was not significant.

Backrubber applications of 5% Ruelene as an oil solution failed to control the cattle grub, although calves utilized the Ruelene applicator for 2 months in late summer and early fall. In the same test 2 quarts of 0.5% Ruelene applied at 80 p.s.i. gave 98% control.

Distribution of Foragers from Honey Bee Colonies Placed in the Middle of a Large Field of Alfalfa[1]

M. D. LEVIN, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2]

ABSTRACT

An experiment was conducted in northern Utah with colonies of honey bees (*Apis mellifera* L.) mass-marked for identification in the field. Cordovan strains were used for one group and colonies in the second group were tagged with radioactive phosphorus. The first group, placed in the center of a large field of alfalfa in bloom, oriented mostly to the field on which they were placed, and retained their dispersion pattern over a 2-week period. The second group, placed in the same location a week later, distributed its foragers in much the same way as the first group but showed a tendency to orient farther afield.

Earlier studies (Levin 1959) on the distribution of field bees in alfalfa indicated that foragers work close to their hives. In the process of obtaining this information, other questions arose concerning the foraging activity of the experimentally marked bees.

In 1957 an experiment was designed to test two hypotheses developed from some of these questions. It was thought (1) that the field bees of a small apiary might restrict their activity almost entirely to the field in which the colonies were placed, and (2) that the distribution pattern of an apiary of marked bees would remain relatively constant for several weeks. In addition, the distribution of foragers from two groups of marked colonies placed on the same site at different times was compared.

METHODS.—The experiment centered on a square field near Plymouth, Utah, (Field A, fig. 1) comprising approximately 150 acres of fairly uniform alfalfa left for second-crop seed. All the alfalfa in the general area was left for second-crop seed, except for Field B, immediately to the east, which was managed for first-crop seed and was well past its bloom. Field C, immediately to the northeast, was cut during the experiment because of a severe armyworm (*Pseudaletia unipuncta* (Haw.)) infestation.

Colonies of bees were marked in two ways. One group consisted of the genetically marked strain known as cordovan and the second group of Caucasian bees tagged with radioactive phosphorus (P^{32}) (Levin 1960).

On July 24, twenty cordovan colonies were moved to the center of Field A. On August 6, ten colonies of black Caucasian bees were placed alongside the cordovans, and on August 7 they were fed radioactive sugar sirup.

On July 30, one week after the cordovans were moved in, samples of bees were collected for 3 days to determine the distribution pattern just before the peak of bloom and before several commercial apiaries were established in the area. Further collections were made, commencing August 8, after the second group of colonies had been moved into the field and made radioactive.

Sampling stations were established in the directions and at the distances indicated in figure 1. The bee collections and population counts were made by three observers, each assigned to a group of sampling stations. Population counts were made each day on 10 separate square-yard areas in the vicinity of each station.

RESULTS AND DISCUSSION.—The data obtained on the central field (A) are summarized in table 1. The average number of cordovans per square yard remained almost the same during both sampling periods. The average per cent of cordovans decreased 39% and the average number of bees per square yard increased 38%. When the radioactive bees were added to the population, they comprised 6.9% of the bees collected.

During the first period the cordovans showed a directional orientation to the east sampling stations. When the radioactive bees were introduced, they also showed the same tendency to work in that direction. The overall population density was slightly higher to the east, but the difference was not significant.

During both periods more marked bees per square yard were found working at the closer stations. This was true in all directions except to the north where the alfalfa bloom was less attractive at the 110-yard distance.

The average numbers of cordovans for the two sampling periods were 0.398 per square yard at 110 yards and 0.147 at 330 yards from the marked colonies. The large decrease per square yard at the greater distance does not reflect a corresponding change in total numbers of cordovans working at each distance. The number working in a 1-yard circular strip at each distance increased slightly from 275.2 in 691 square yards to 304.9 in 2,072 square yards. There were 187.3 radioactive bees working 110 yards from their colonies and 115.5 at 330 yards.

The data obtained from sampling stations on nearby fields are summarized in table 2. The increase in bee populations observed on the central field was not duplicated at these stations. The cordovans made up negligible percentages of foragers except toward the east, where at the two nearest distances they comprised more than 5% of the population, thus supporting the first hypothesis.

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[2] W. P. Nye and R. A. Nielsen assisted in moving the colonies and collecting the data.

Table 1.—The distribution of marked honey bees on the central field during two sampling periods.

Direction and Distance (yards)	Collected June 30 to Aug. 1			Collected August 8 to 13				
	Number of Bees per Square Yard		Per Cent Cordovan (a)	Number of Bees per Square Yard			Per Cent Cordovan (a)	Per Cent Radioactive (c)
	Total (b)	Cordovan (d)		Total (b)	Cordovan (d)	Radioactive (e)		
West								
110	1.4	0.339	31.9	2.4	0.426	0.039	17.6	1.6
330	1.7	.286	15.6	1.4	.077	.016	6.1	1.3
East								
110	1.6	0.652	38.8	2.8	0.734	0.853	25.8	30.4
330	1.1	.196	17.8	1.5	.124	.076	8.4	5.1
North								
110	0.7	0.226	37.1	0.9	0.172	0.089	19.4	4.2
330	1.2	.159	14.7	2.0	.176	.106	8.9	5.3
South								
110	1.3	0.253	22.1	2.0	0.384	0.102	19.1	5.1
330	1.4	.087	5.7	1.4	.073	.025	6.9	1.9
Average	1.3	.275	22.96	1.8	.271	.163	14.025	6.86

Analysis of Variance

Source	df	Mean Squares (a)	(b)	df	(c) Mean Squares
Sampling period	1	320.4**	1.614**	—	—
Direction	3	62.2	0.462	3	419.7**
Distance	1	1,022.4**	.733*	1	539.4**
Direction×Distance	3	5.1	.186	3	264.9**
Error	7	18.8	.084	24	18.4
Total	15			31	

	(d)		(e)	
	df	Mean Squares	df	Mean Squares
Sampling period	1	0.0002	—	—
Dates	5	.0187	3	0.0125
Direction	3	.1724**	3	.3286**
Distance	1	.9480**	1	.3689**
Direction×distance	3	.1591**	3	.2837**
Error	42	.0235	21	.0147*
Total	55		31	

* Significant at 5% level.
** Significant at 1% level.

However, the number of cordovans decreased considerably during the second period. A few more radioactive bees worked on these fields, and they too showed a continuance of the eastward orientation observed on the central field.

The distribution pattern of the cordovan bees did not change much during the 2-week period even though the available forage was decreased by 100 acres owing to cutting Field C and the overall bee population in the area was increased by about 70 colonies. Raising the population density on the central field did not affect the orientation of the cordovans, thus providing evidence supporting the second hypothesis. The proportion of cordovans to total number of bees decreased by almost the same percentage that the overall population density increased.

The population density showed a gradient in relation to the apiary only during the second sampling period, when the bee population was highest. This is in accord with an earlier observation (Levin *et al.* 1960).

The distribution of the radioactive bees was similar to that of the cordovans. Since there were only 10 of these colonies compared with 20 cordovans, only half as many tagged bees were recovered. They were collected a little farther from their colonies than were the cordovans because they probably oriented to forage that came into bloom after the initial orientation of the cordovans.

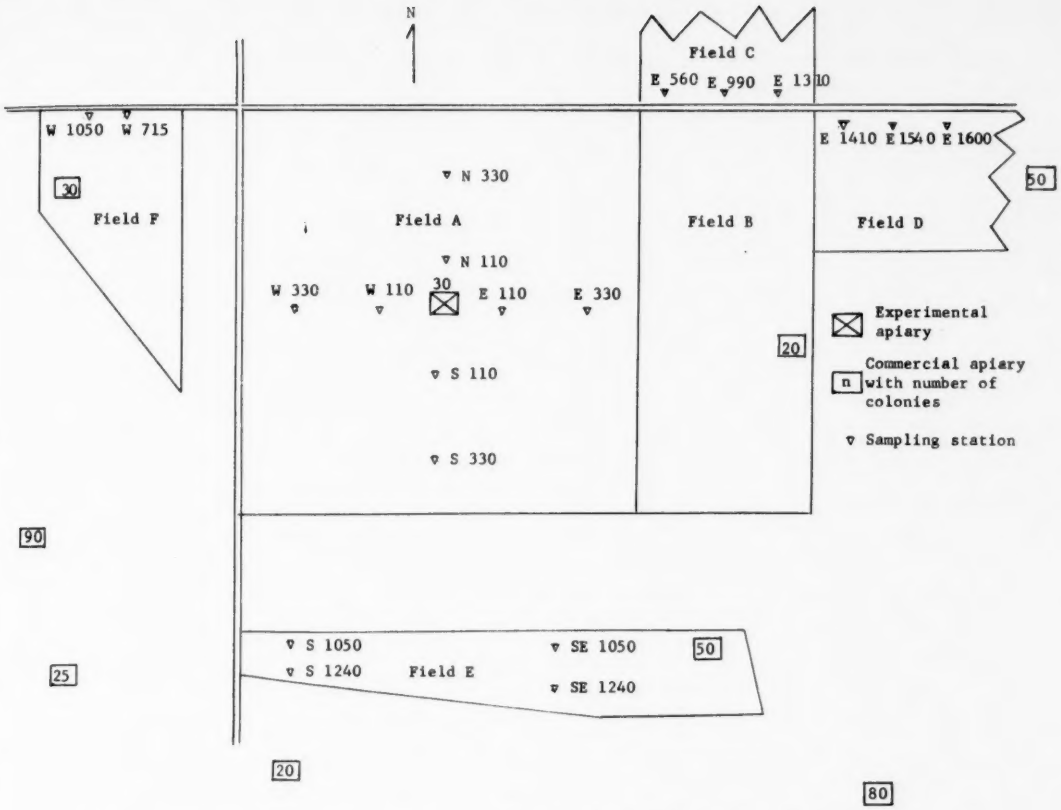

FIG. 1.—Location and distance (in yards) of sampling stations in alfalfa fields, Plymouth, Utah. (Not to scale)

Table 2.—The distribution of marked honey bees on neighboring fields during two sampling periods.

Direction and Distance (yards)	Collected June 30 to Aug. 1			Collected August 8 to 13				
	Number of Bees per Square Yard		Per Cent Cordovan	Number of Bees per Square Yard			Per Cent Cordovan	Per Cent Radioactive
	Total	Cordovan		Total	Cordovan	Radioactive		
South								
1,050	1.8	0.025	1.1	1.70	0.024	0	1.33	0
1,240	1.8	.042	2.4	3.10	.012	0.004	0.36	0.17
Southeast								
1,050	2.4	0.012	0.4	—	—	—	—	—
1,240	2.1	.020	1.0	—	—	—	—	—
West								
715	2.2	0.080	3.8	0.87	0.007	0.004	0.81	0.55
1,050	1.9	.023	1.0	.75	.003	.002	.52	.39
East								
560	0.4	0.009	9.7	—	—	—	—[a]	—
990	.5	.024	5.7	—	—	—	—[a]	—
1,310	1.0	.023	1.8	—	—	—	—[a]	—
1,410	1.7	.0	0	1.00	0.004	0.026	0.58	2.65
1,540	—	—	—	1.05	.009	.037	.89	3.57
1,600	—	—	—	1.50	.001	.051	.17	3.10
Average	1.58	0.0258	2.69	1.42	0.0086	0.018	0.67	1.49

[a] Field cut between sampling periods.

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Control of Face Flies (*Musca autumnalis*) on Beef Cattle in Indiana[1]

R. C. DOBSON and D. A. HUBER,[2] *Purdue University, Lafayette, Indiana*

ABSTRACT

Experiments utilizing organic insecticides on cable-rubber devices for the control of face fly, *Musca autumnalis* DeGeer, were conducted in 1959 and 1960. Populations were adequately reduced by methoxychlor, toxaphene, DDT and ronnel. Five per cent Crag Fly Repellent® (butoxy polypropylene glycol), DDVP and dimethoate were ineffective in reducing populations.

Musca autumnalis DeGeer was first reported in North America by Vockeroth (1953) who recorded it as occurring in Nova Scotia in 1952. Since that time, it had spread by 1959 as far south as Virginia and as far north and west as Wisconsin. By July 15, 1960, it had spread further west and south into Missouri and Iowa (U. S. Department of Agriculture 1960).

During 1959 and 1960, this insect proved to be a serious pest of livestock in Indiana, particularly cattle, but also affected horses and ponies as reported by Matthew & Dobson (1960). Experiments were conducted during the summer of 1959 and again in 1960 against this pest.

MATERIALS AND METHODS.—Some of the common chlorinated hydrocarbon insecticides were tested against this pest both as sprays and as oil solutions on cable-rubber devices. The first test was set up on the Miller-Purdue Memorial Farm at Upland, Indiana on July 8, 1959. At this time, 176 beef animals were divided into two lots. One lot was sprayed with DDT and the other with methoxychlor. Both materials were applied at the rate of 4 pounds actual toxicant per 100 gallons of water. The formulations used were wettable powders containing 50% of the active ingredient. A second DDT spray of the same concentration was again applied on July 29 to 49 animals. Following this second spraying, the remaining untreated animals were divided into lots and placed, four per pasture, in 32 pastures which ranged from 1 to 5 acres in size. A swing-type cable-rubber device was placed in each of these pastures (Dobson & Flint 1953). Unfortunately, all of the rubbers were impregnated with 5% DDT in No. 2 diesel oil on July 29, and were rendered unfit for the use of other materials.

On June 30, 1960, a series of insecticide trials were started at the Miller-Purdue Memorial Farm. The first series consisted of the application of six materials replicated four times in 24 small pastures ranging from 0.99 to 5 acres in size (see figure 1, Arabic numbered pastures). They contained 4 to 8 animals each. The cable rubbers were the same as those used the previous year but they were re-wrapped and repaired before the materials were applied. Each rubber was treated on June 30, and again on July 15, following rains totaling 3½ inches on July 12–13. Two heavy showers occurred between July 20 and July 28. Counts of flies were taken at intervals utilizing a 7×35 field binocular after approaching on foot as closely as the relatively tame cattle would allow. The number of flies on the head of each animal in the pasture was counted and the average for the plot was calculated. The following materials were used in this trial: (1) 5% methoxychlor in No. 2 diesel oil; (2) 5% toxaphene in mineral seal oil; (3) 5% DDT in mineral seal oil; (4) 1% ronnel in No. 2 diesel oil; (5) 5% DDT+3% Tabatrex® (dibutyl succinate) in No. 2 diesel oil; and (6) 5% DDT in No. 2 diesel oil.

Another test utilizing 12 pastures of 4 acres each was set up on this same farm. These pastures contained from 5 to 10 head of yearling steers. This experiment was started on July 9, with the rubbers being retreated on July 20, after the counts were taken and following 3½ inches of rain on July 12–13. Two heavy local showers occurred between the July 20 application and July 28 when one set of counts was taken. These 12 pastures were in the same large field as were the 24 smaller pastures, the only difference being in size. Four materials were tested and each was replicated three times. The plots were laid out as shown in figure 1 (see Roman numerals). The materials applied to this set of plots were (I) 5% DDT in mineral seal oil used as a treatment check; (II) 5% Crag Fly Repellent® (butoxy polypropylene glycol) in mineral seal oil; (III) 1% DDVP in mineral seal oil; (IV) 1% dimethoate in mineral seal oil.

RESULTS.—*Experiment 1.*—In this experiment (1959) control of the face fly with both DDT and methoxychlor was excellent within 24 hours following spraying on July 8. This control lasted 14 days in the case of DDT and 10 days with methoxychlor. A subsequent DDT spray applied on July 29 applied to 49 head of cattle gave approximately 14 days control of the face fly but was inadequate in controlling horse flies (Tabanidae) which were a problem at that time. On July 29, all pastures with cable rubbers in them, E-1 through E-9, F-1 through F-9, K-1 through K-7 and L-3 through L-12 (see figure 1) were treated with 5% DDT in diesel oil. The populations before treatment ranged about 45 to 50 per head which

[1] Purdue University Agricultural Experiment Station Journal Paper No. 1720.

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[2] Department of Entomology and Purdue University Experiment Station Farms, respectively.

Table 1.—Effectiveness of six insecticide formulations applied to cable rubbers against the face fly. Miller-Purdue Memorial Farm—South Field, 1960.

Formulation[a]	Plot No.	Average Number of Flies[b] June 30[c] Per Calf	June 30[c] Per Treatment	July 20 Per Calf	July 20 Per Treatment	July 28 Per Calf	July 28 Per Treatment	August 12 Per Calf	August 12 Per Treatment
5% methoxychlor in No. 2 diesel oil	E-1	11.4	10.8	0.6	3.2	2.6	4.2	8.2	8.5
	F-4	10.0		2.5		3.4		5.8	
	F-7	11.7		5.0		5.2		5.6	
	K-3	10.2		4.5		5.4		14.2	
5% toxaphene in mineral seal oil	E-2	9.5	10.2	0.3	4.9	9.5	6.3	11.0	7.2
	F-2	8.0		4.8		2.2		8.8	
	F-6	12.4		8.0		6.2		5.0	
	E-9	10.7		6.7		7.4		3.8	
5% DDT in mineral seal oil	E-3	11.2	9.1	0.8	2.6	11.5	10.2	7.5	8.7
	E-7	12.0		4.5		9.0		10.5	
	K-1	5.0		0.3		2.5		9.2	
	K-6	8.2		4.7		17.8		7.5	
1% ronnel in No. 2 diesel oil	E-4	11.2	12.3	4.2	5.5	12.7	10.3	15.5	7.1
	F-5	13.0		3.7		9.6		5.6	
	F-9	14.8		8.8		12.3		—	
	K-4	10.0		5.2		6.5		7.2	
5% DDT+Tabatrex in No. 2 diesel oil	E-5	12.5	9.3	2.5	1.9	2.6	5.8	4.4	6.0
	F-3	10.6		2.0		3.5		3.4	
	K-2	4.7		1.2		2.0		3.0	
	K-7	—		—		15.0		13.2	
5% DDT in No. 2 diesel oil	E-6	8.6	10.7	4.2	3.8	10.2	8.9	5.5	7.6
	F-1	11.8		4.2		5.0		6.2	
	F-8	8.7		2.8		5.2		9.5	
	K-5	13.6		4.0		15.0		9.0	
Checks[d]					14.7		17.5		26.6

[a] Treated June 30; 3.5 inches of rain July 12–13. Retreated July 15; two heavy showers between July 20 and July 28.
[b] Average based on 4 to 8 calves. [c] Pretreatment counts. [d] Figures based on 4 calves per plot — 10 plots.

Table 2.—Effectiveness of four insecticides applied to cable rubbers against the face fly. Miller-Purdue Memorial Farm—North Field, 1960.

Treatment and Per Cent Concentration[a]	Plot No.	Average Number of Flies per Calf July 20[b]	July 28	August 12
DDT, 5	D-5	10.0	10.2	10.0
	B-2	7.6	6.9	8.8
	B-5	8.6	5.0	9.8
Treatment average		8.7	7.3	9.5
Crag Fly Repellent, 5	C-1	35.0	43.7	34.0
	B-1	11.0	17.5	13.7
	B-4	19.0	9.6	32.0
Treatment average		21.6	23.6	26.6
DDVP, 1	D-3	17.0	29.0	21.0
	C-3	14.0	15.0	23.3
	A-4	15.0	26.2	35.0
Treatment average		15.3	23.4	26.4
Dimethoate, 1	D-4	23.0	29.0	30.0
	C-2	12.0	19.1	30.0
	A-1	23.0	32.1	20.6
Treatment average		19.3	26.7	26.9

[a] All materials carried in mineral oil. Treated July 9. 3.5 inches of rain July 12–13. Retreated July 20. Two heavy showers between July 20 and July 28.
[b] Counts taken before retreatment.

dropped for the remainder of the season to 4 to 6 per animal.

Experiment 2.—In the 1960 experiment utilizing 24 pastures (see figure 1, Arabic numerals), readings were taken before the materials were applied on June 20 and again on July 20, July 28 and August 12. The data are given in table 1. Populations on untreated animals averaged between 14.7 and 26.6 for this period.

Data in table 1 show that all materials gave substantial reductions in the average number of flies per head. Ronnel was the least effective material on July 20 and July 28 but was adequate on August 12. The formulation containing DDT and Tabatrex was consistently effective and was the best material according to fly counts at the beginning and end of the test. The formulation of DDT in mineral seal oil was comparable to the diesel oil formulations on the first count but was consistently poorer in the subsequent tabulations. Five per cent methoxychlor in No. 2 diesel oil gave excellent control on the first and second counts but apparently was losing its effectiveness on the third count A formulation of 5% toxaphene gave excellent results throughout the period of the test.

Experiment 3.—In this experiment (1960) on the north half of the large field of the Miller-Purdue Memorial Farm (see fig. 1), of the four insecticides used, only 5% DDT gave control. The other three insecticides did not

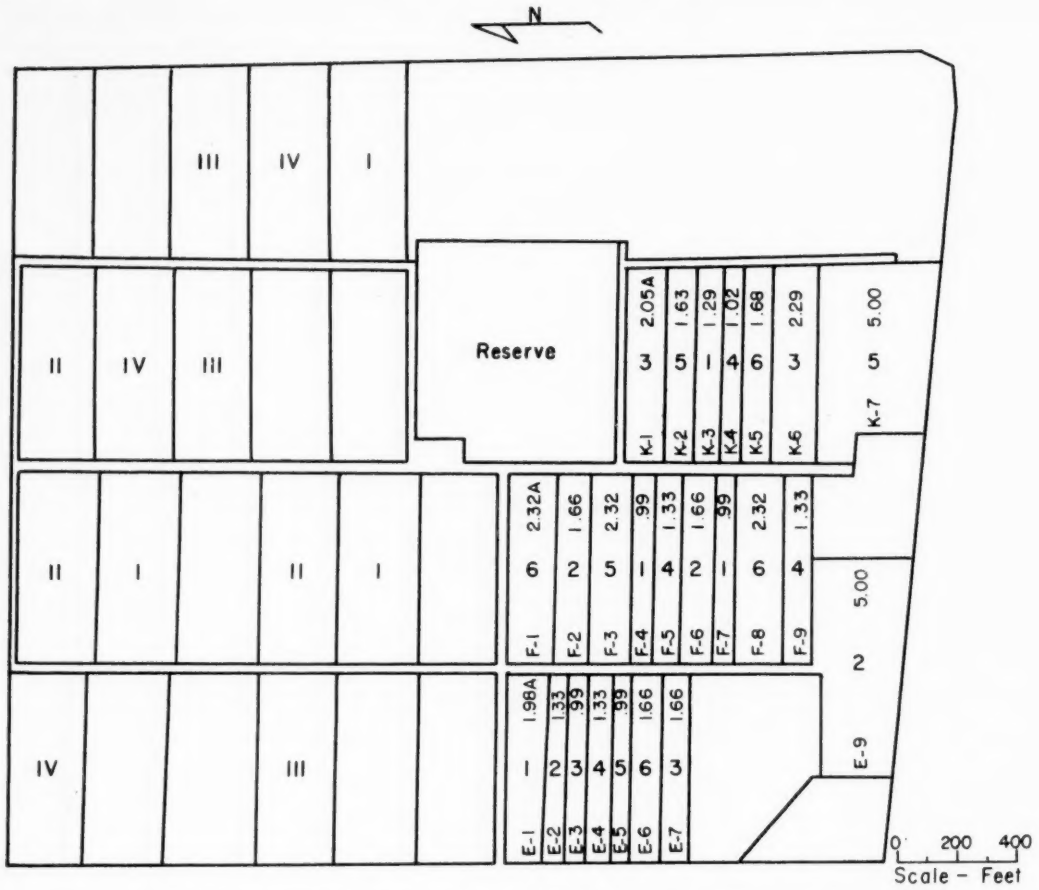

FIG. 1.—Arrangement of cable-rubber plots. Miller-Purdue Farm. Grant County, Indiana. 1960.

give control as is evidenced by the average number of flies per animal (table 2). Those plots, where control was unsatisfactory, were visible upon first examination since the animals remained bunched up, and fought flies throughout the day while those cattle in the fields with DDT-treated rubbers grazed normally.

CONCLUSIONS.—Results based on 2 years of experimental data show that face flies can be controlled in pastures using cable-rubber devices. Materials such as methoxychlor, toxaphene and DDT are effective in reducing populations of this pest when used with these devices. In these tests there was no advantage in using mineral seal oil over No. 2 diesel oil. Ronnel reduced the populations adequately, but was no better than the DDT formulations. Five per cent Crag Fly Repellent, 1% DDVP and 1% dimethoate applied to cable-rubber devices were each ineffective in reducing face fly populations.

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Toxicity of Some Insecticides to the Red Flour Beetle, *Tribolium castaneum*[1]

N. Shi, G. C. Sengupta and B. N. Satpathy, *State Agricultural Research Station, Bhubaneswar, Orissa, India*

Abstract

A laboratory experiment was conducted in 1960 comparing the toxicity of parathion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate), endrin, malathion, dieldrin and DDT to *Tribolium castaneum* (Herbst) (Tenebrionidae—Coleoptera), a storage pest. The insecticides were formulated in the laboratory from the technical grades and used in the form of emulsion. Benzene and Triton X-100 were used as solvent and emulsifier, respectively, and their proportion in the final formulation being kept as 5% w/v of the solvent and 0.625% w/v of the emulsifier. Approximately 5,000 adult beetles reared in the laboratory were kept in petri dishes and were sprayed directly under Potter's tower. Observations on mortality were recorded 72 hours after spraying. When the values of L.C.50 were taken into consideration the order of toxicity of different insecticides were: parathion>Diazinon>endrin >malathion>dieldrin>DDT. Thus parathion, Diazinon, endrin, malathion and dieldrin were found to be more toxic than DDT, *i.e.*, 84.1, 62.4, 17.3, 14.8 and 2.3 times, respectively, as toxic as DDT.

The advent of the toxic properties of DDT provided a major stimulus to the search for new synthetic organic chemicals, as a result of which most important chlorinated hydrocarbon insecticides (BHC, chlordane, toxaphene, aldrin, dieldrin, endrin, et cetera) and organophosphorus insecticides (parathion, malathion, Diazinon, TEPP, et cetera) and other allied compounds have been evolved in various countries. Experiments to assess the potency of different insecticides for controlling insect pests have been conducted by various workers in the field as well as in the laboratory. From all those experiments it has been known that, in general, every insecticide exhibits a certain degree of specificity of action insofar as its effects on different species and orders of insects are concerned. Hence various research workers have studied these insecticides in order to list them according to their relative toxicity to control insect pests belonging to different species, as the results are urgently needed for the guidance of those engaged in practical control operations in the field. The importance of bioassay studies in the laboratory has been explained previously by Pradhan & Bhatia (1952). The present investigation reports the results of bioassay studies with six insecticides, parathion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate), endrin, malathion, dieldrin, and DDT tried against the red flour bettle *Tribolium castaneum* (Herbst).

Materials and Methods.—The form in which these insecticides were received and the source from which they were obtained are as follows: parathion (pure) was received from Messrs. Chika Private Ltd., Bombay; malathion (95%) was obtained from Lederle Laboratories India Private Ltd., Bombay; endrin (91%) and dieldrin (96%) were supplied by Messrs. Burmah-Shell; Diazinon technical (100%) was obtained from Tata-Fison Private Ltd., Bombay and pure p,p'-DDT was crystallized in the laboratory from a commercial product containing 80% of this isomer. All these technical insecticides were used in the form of emulsion, benzene and Triton X-100 (supplied by Messrs. Amritalal & Co., Bombay) being used as solvent and emulsifier, respectively, their proportion in the final formulation being kept at 5% (w/v) and 0.625% (w/v), respectively. The insecticides used were formulated in our laboratory from the technical grades of insecticides supplied. Allowance was made for impurities in the technical grades while preparing the stock solution and required concentrations were prepared out of stock solution by adding the required amount of emulsified water.

The spraying in the laboratory was done with a tower sprayer based on the principle of Potter's tower (Potter 1941), using one milliliter of spray liquid for each spray operation. The pressure during spraying was maintained at 42.5 cm. of mercury and the average deposit of emulsion was 0.016 mg. per sq. cm. The test insects were kept in petri dishes (7 cm. diameter) sprayed directly under the spray tower. The insects were allowed to remain in the same petri dishes till observations on mortality were recorded 72 hours after spraying, as was done by Burkhalter and Arant (1960). The insects were examined one by one with the naked eye, and also under a binocular microscope when necessary, and were divided into the following five categories as described by Pradhan (1949) and Tattersfield & Potter (1943): normal, slightly affected, badly affected, moribund, and dead. For calculation of the L.C.50, moribund insects were added to the category of the dead.

Results and Discussion.—The mortality data noted 72 hours after treatment were subjected to probit analysis (Finney 1952) and the results are presented in table 1 and figure 1.

The figures on relative toxicity have been worked out using L.C.50 as a criterion and taking that of DDT as one. When the values of L.C.50 were taken into consideration the order of toxicity of different insecticides was found to be: parathion > Diazinon > endrin > malathion > dieldrin > DDT. Thus parathion, Diazinon, endrin, malathion and dieldrin were found to be more toxic than DDT, *i.e.*, 84.1, 62.4, 17.3, 14.8 and 2.3 times, respectively, as toxic as DDT. The results of Pradhan & Bhatia (1956) showing that *Tribolium castaneum* was more susceptible to dieldrin than DDT are in agreement with the results obtained in the present investigation although the insecticides were applied in a different manner.

(*Table 1 and Figure 1 on next page*)

[1] Accepted for publication August 18, 1960.

Table 1.—Comparative toxicity of certain insecticides to *Tribolium castaneum*.

INSECTICIDES	HETEROGENEITY AT P=0.05[a]	REGRESSION EQUATIONS	L.C. 50	FIDUCIAL LIMITS OF L.C. 50	RELATIVE TOXICITY
Parathion	$\chi_{(4)}^2=1.8967$	Y=1.5125X+3.5383	0.00009255	+0.00009367 −0.00009143	84.1
Diazinon	$\chi_{(4)}^2=0.3445$	Y=1.9323X+2.8837	0.0001246	+0.0001257 −0.0001235	62.4
Malathion	$\chi_{(4)}^2=5.2022$	Y=2.2576X+1.1186	0.0005240	+0.0005253 −0.0005227	14.8
Endrin	$\chi_{(4)}^2=2.1194$	Y=3.3618X−0.5559	0.0004494	+0.0004506 −0.0004482	17.3
DDT	$\chi_{(4)}^2=1.0240$	Y=1.5289X+0.5796	0.007784	+0.007799 −0.007769	1.0
Dieldrin	$\chi_{(4)}^2=4.5182$	Y=2.7999X−2.1085	0.003457	+0.003458 −0.003456	2.3

[a] In none of these cases, the data were found to be significantly heterogeneous at P=0.05; Y=Probit kill; X=log. (concentration×10^5); L.C. 50=Concentration calculated to give 50% mortality; $\chi_{(4)}^2$=Chi−square test.

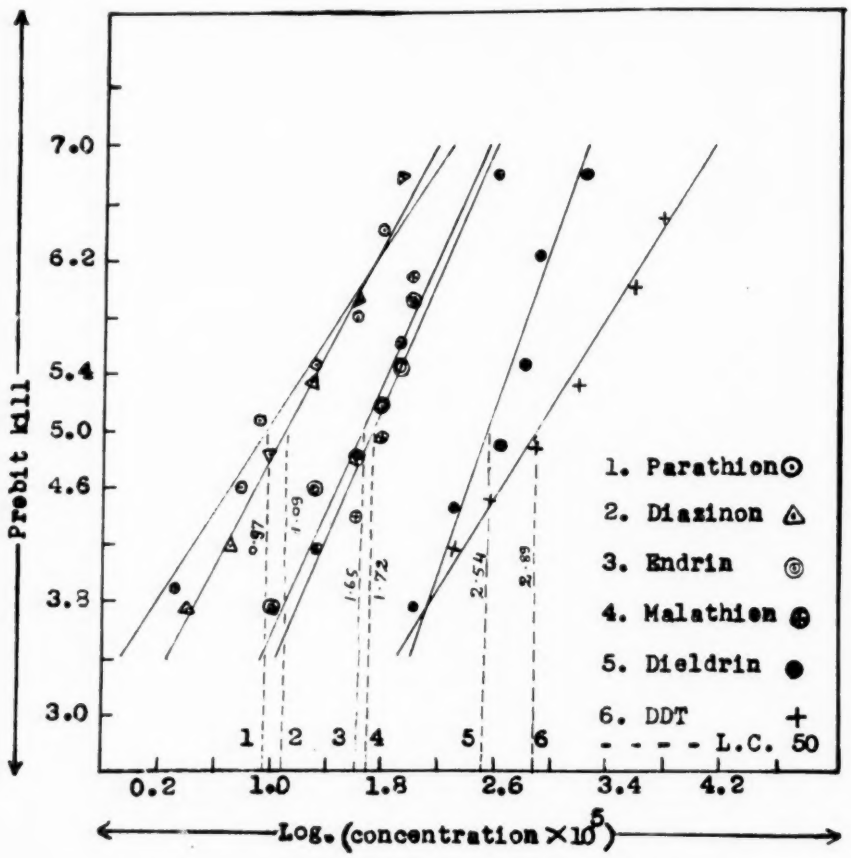

FIG. 1.—Comparative toxicity of different insecticides to *Tribolium castaneum*.

RELATIVE TOXICITY

84.1

62.4

14.8

17.3

1.0

2.3

= Concentration

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Methods of Delaying Selection of Acaricide Resistant Strains of the European Red Mite[1]

DEAN ASQUITH,[2] *The Pennsylvania State University, Fruit Research Laboratory, Arendtsville, Pa.*

ABSTRACT

Certain acaricides were tested on apple for three seasons as control agents for the European red mite, *Panonychus ulmi* (Koch), and the two-spotted spider mite, *Tetranychus telarius* (L.), according to three plans: (1) single acaricides alone at standard concentration in consecutive acaricidal applications; (2) two acaricides separately at standard concentration in alternate applications; (3) two acaricides mixed, with each at one-half standard concentration, in consecutive applications. In the third season of continuous use according to plan 1, the acaricides failed to control the European red mite, an indication that the use of each chemical had developed a strain of this pest resistant to it. The same acaricides continued to give a high degree of control of this pest in the third season of continuous use according to plans 2 and 3.

Previously, Asquith (1960) reported the results of testing certain acaricides on apple against the European red mite, *Panonychus ulmi* (Koch), and the two-spotted spider mite, *Tetranychus telarius* (L.), in 1958 and 1959. Certain acaricidal treatments, which were identified with specific trees in both years, were applied according to the following three plans: (1) single acaricides alone at standard concentration in consecutive applications; (2) two acaricides separately at standard concentration in alternate applications, and; (3) two acaricides mixed, with each at one-half standard concentration, in consecutive applications. This earlier report presented data that indicated certain advantages of plan 3. At the same time, it stated that up to the end of the 1959 season the experiment had not yielded any data to fulfill the original objective which was to determine if the three methods of using acaricides outlined above might develop resistant strains of mites at different rates. Data from the test in 1960 reported herein supply at least part of the answer.

MATERIALS AND METHODS.—Information regarding the design of the experiment and the method of handling the data will be found in the previous paper. The identity of the acaricidal treatments with specific trees was maintained in 1960.

For the sake of clarity, it is necessary to present the data on the mites for 1958, 1959 and 1960 in table 1. Hence, in view of the space limitations in table 1, explanations of the acaricidal treatments are listed below. The weights of the acaricides are the amounts used per 100 gallons of spray.

Treatment 1

1958. Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite), 2 pounds of wettable powder (15% active) applied on July 8 and July 23.

1959. Dimethoate wettable powder (50% active) at the rate of 8 ounces on June 19, June 30 and July 24.

1960. Dimethoate wettable powder (25% active) at the rate of 1 pound on June 15 and 1.5 pounds on June 30 and July 22.

Treatment 2

1958. Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone), 8 ounces of wettable powder (25% active), on July 8 and July 23.

1959. Tedion WP, 8 ounces on June 19, June 30 and July 24.

1960. Tedion WP, 12 ounces on June 15, June 30 and July 22.

Treatment 3

1958. Tedion WP, 4 ounces, mixed with Aramite WP, 1 pound, on July 8 and July 23.

1959. Tedion WP, 4 ounces, mixed with dimethoate WP (50% active), 4 ounces, on June 19, June 30 and July 24.

1960. Tedion WP, 6 ounces, mixed with dimethoate WP (25% active), 8 ounces, on June 15 and with dimethoate WP (25% active), 12 ounces, on June 30 and July 22.

Treatment 4

1958. Aramite WP, 2 pounds, on July 8 alternated with Tedion WP, 8 ounces, on July 23.

1959. Dimethoate WP (50% active), 8 ounces, on June 19, Tedion WP, 8 ounces, on June 30, and dimethoate WP (50% active), 8 ounces, on July 24.

1960. Tedion WP, 12 ounces, on June 15, dimethoate WP (25% active), 12 ounces, on June 30, and Tedion WP, 12 ounces, on July 22.

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[2] Acknowledgment is made of the valued assistance of Guy A. Herring, W. L. Coradetti and Peter D. Asquith.

Table 1.—Control of a mixed population of the European red mite and the two-spotted spider mite based on the average number of mites per leaf accumulated in postspray counts from five replicates of each treatment in 1958, 1959 and 1960.[a]

Treatment Number	Year and Acaricides	Method of Use	Postspray Average Accumulated Mites/Leaf[b]		
			1958	1959	1960
1	1958 Aramite 1959 Dimethoate 1960 Dimethoate	Alone Alone Alone	20 (87)	23 (93)	50 (80)
2	1958 Tedion 1959 Tedion 1960 Tedion	Alone Alone Alone	29 (82)	54 (82)	176 (29)
3	1958 Aramite, Tedion 1959 Dimethoate, Tedion 1960 Dimethoate, Tedion	Mixed Mixed Mixed	16 (90)	26 (91)	21 (92)
4	1958 Aramite, Tedion 1959 Dimethoate, Tedion 1960 Tedion, dimethoate	Alternate Alternate Alternate	19 (88)	32 (90)	48 (81)
5	1958 Kelthane 1959 Kelthane 1960 Kelthane	Alone Alone Alone	9 (95)	14 (95)	86 (65)
6	1958 Kelthane, Aramite 1959 Kelthane, dimethoate 1960 Kelthane, dimethoate	Mixed Mixed Mixed	8 (95)	13 (96)	26 (89)
7	1958 Kelthane, Aramite 1959 Kelthane, dimethoate 1960 Dimethoate, Kelthane	Alternate Alternate Alternate	22 (86)	17 (94)	32 (87)
8	1958 Kelthane, Tedion 1959 Kelthane, Tedion 1960 Kelthane, Tedion	Mixed Mixed Mixed	11 (93)	24 (92)	12 (95)
9	1958 Kelthane, Tedion 1959 Kelthane, Tedion 1960 Tedion, Kelthane	Alternate Alternate Alternate	19 (88)	29 (90)	29 (88)
10	Check (No acaricides in 1958, 1959, 1960)		156	303	247
	L.S.D. at 5% level at 1% level		2.7 3.6	10.4 14.0	31.2 41.9

[a] The European red mite never constituted less than 98.5 % of the population in any treatment during 1960.
[b] Per cent control given in parenthesis.

Treatment 5

1958. Kelthane® (1,1 bis (*p*-chlorphenyl)-2,2,2-trichloroethanol), 2 pounds of wettable powder (18.5% active) on July 8 and July 23.
1959. Kelthane WP, 2 pounds, on June 19, June 30 and July 24.
1960. Kelthane WP, 2 pounds, on June 15, June 30 and July 22.

Treatment 6

1958. Kelthane WP, 1 pound, mixed with Aramite WP, 1 pound, on July 8 and July 23.
1959. Kelthane WP, 1 pound, mixed with dimethoate WP (50% active), 4 ounces, on June 19, June 30 and July 24.
1960. Kelthane WP, 1 pound, mixed with dimethoate WP (25% active), 8 ounces, on June 15 and with dimethoate WP (25% active), 12 ounces, on June 30 and July 22.

Treatment 7

1958. Kelthane WP, 2 pounds, on July 8 alternated with Aramite WP, 2 pounds, on July 23.
1959. Kelthane WP, 2 pounds, on June 19, dimethoate WP (50% active), 8 ounces, on June 30, and Kelthane WP, 2 pounds, on July 24.
1960. Dimethoate WP (25% active), 1 pound, on June 15, Kelthane WP, 2 pounds, on June 30, and dimethoate WP (25% active), 1.5 pounds, on July 22.

Treatment 8

1958. Kelthane WP, 1 pound, mixed with Tedion WP, 4 ounces, on July 8 and July 23.
1959. Kelthane WP, 1 pound, mixed with Tedion WP, 4 ounces, on June 19, June 30 and July 24.
1960. Kelthane WP, 1 pound, mixed with Tedion WP, 6 ounces, on June 15, June 30 and July 22.

Treatment 9

1958. Kelthane WP, 2 pounds, on July 8 alternated with Tedion WP, 8 ounces, on July 23.
1959. Kelthane WP, 2 pounds, on June 19, Tedion WP, 8 ounces, on June 30, and Kelthane WP, 2 pounds, on July 24.
1960. Tedion WP, 12 ounces, on June 15, Kelthane WP, 2 pounds, on June 30, and Tedion WP, 12 ounces, on July 22.

Treatment 10 (Check)

1958, 1959 and 1960. No acaricides.

DISCUSSION AND CONCLUSIONS.—In 1960, the European red mite made up at least 98.5% of the mite population on the treated trees. The poor results obtained with Tedion, treatment 2, and Kelthane, treatment 5, as shown for 1960 in table 1 were, therefore, against this species. In table 1, the most important point to observe is the excellent control obtained with treatment 8, the Kelthane-Tedion mixture, and with treatment 9, Kelthane alternated with Tedion, in 1960, the third season of use on the same trees in spite of the failure of these same acaricides as used in treatments 2 and 5, *i.e.*, alone for three seasons.

The data obtained indicate that both Kelthane and Tedion, when used separately as the only acaricides for a period of three seasons, caused the development of strains of the European red mite resistant to each, respectively. The data also show that when these acaricides were used alternately at standard concentration or mixed together, with each at one-half standard concentration, on the same trees for 3 years, strains of the European red mite did not develop with enough resistance to impair the control obtained during the third year of use. The superiority of the latter two methods of employing acaricides over the former is clearly indicated.

COMMENT.—It is planned to continue this experiment to determine whether resistant strains of the European red mite will develop from alternate applications of Kelthane and Tedion or by the mixture of Kelthane and Tedion. It should also be possible to determine whether the mites that are resistant to Kelthane are also resistant to Tedion and vice versa.

Further work with mixtures of unrelated chemicals as control agents for mites and insects is suggested on the basis of the premise that selection of these animals for multiple characters is usually a slower process than their selection for single characters.

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In Vitro Stability and Recovery of Insecticides from Milk[1]

J. A. TIMMERMAN, JR., H. W. DOROUGH, J. R. BUTTRAM, and B. W. ARTHUR
Department of Zoology-Entomology, Auburn University, Auburn, Alabama

ABSTRACT

Recoveries of Sevin® (1-naphthyl *N*-methylcarbamate), Kepone® (decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta [cd] pentalen-2-one), Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), Bayer 22408 (*O*-naphthalimido-*O,O*-diethyl phosphorothioate), Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate), and CoRal® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate) from milk ranged from 87% to 97% using acetonitrile and chloroform as the primary extraction solvents and acetonitrile-*n*-hexane as a cleanup procedure. This extraction procedure was somewhat less effective for removing known amounts of DDT and dieldrin from milk. Acetonitrile-chloroform extraction of milk was usually more efficient for removing insecticides than was a 2:1 mixture of diethyl ether and *n*-hexane. Ruelene, Bayer 22408, and Bayer 29493 were stable *in vitro* in raw milk for periods up to 14 days of incubation, but after 3 days incubation, some Co-Ral equivalents remained in the milk residue following acetonitrile-chloroform extraction.

Uniform methods for extracting insecticide residues from milk are lacking. Existing methods are somewhat variable in efficiency. The analysis of milk involves a change in extraction solvents and cleanup procedures for almost every insecticide. Freeze drying milk and extraction of the solids with petroleum ether followed by acetonitrile partitioning of the petroleum ether extracts resulted in 63% to 70% recovery of Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate) (Blinn & Gunther 1955), and a similar procedure involving carbon tetrachloride extraction of the milk solids resulted in 79% to 132% recovery of malathion (Norris *et al.* 1958). Incomplete recovery of parathion (Gyrisco *et al.* 1959) was obtained when milk was extracted directly with *n*-hexane; dilution of milk with 95% ethanol and extraction with *n*-hexane was 99% effective in removing Co-Ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate) from milk (Krueger *et al.* 1959), but was only 50% to 60% effective in removing Chlorthion® (*O,O*-dimethyl *O*-(3-chloro-4-nitrophenyl) thiophosphate) (Kolbezen & Barkley 1954). Only 65% of the dimethoate (Dauterman *et al.* 1959) and 40% to 50% of the Phosdrin® (a mixture of 1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate and related compounds) (Casida *et al.* 1958) added to milk were recovered in chloroform extracts.

DDT (Gannon *et al.* 1959) is generally extracted from milk with *n*-hexane and then separated from the butterfat on a sulfuric acid-Celite column, whereas the closely related methoxychlor is removed with the same solvent as DDT but partitioning of the *n*-hexane extracts with nitromethane was used to separate the insecticide from butterfat (Cluett *et al.* 1960). Saponification of milk, *n*-hexane extraction, and magnesium oxide-Celite column chromatography have been used in the analysis of milk for aldrin and dieldrin (Gannon *et al.* 1959), while pentane

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Table 1.—Recovery of Sevin and 1-naphthol from milk using the acetonitrile-chloroform extraction procedure.

P.P.M. SEVIN[a]		PER CENT RECOVERED	P.P.M. 1-NAPHTHOL[a]		PER CENT RECOVERED
Added	Found		Added	Found	
0.1	0.098	98	0.1	0.103	103
.3	.287	96	.3	.297	99
.5	.475	95	.5	.473	95
.8	.743	93	.8	.748	94
1.2	1.131	94	1.2	1.116	93

[a] Averaged from 5 determinations.

Table 2.—Recoveries of insecticides from milk by two extraction procedures.

INSECTICIDE	ACETONITRILE-CHLOROFORM			*n*-HEXANE-ETHER		
	P.p.m. Added	P.p.m. Found	Per Cent Recovery	P.p.m. Added	P.p.m. Found	Per Cent Recovery
Sevin	1.2	1.13	94 (5)[a]	1.2	0.94	78 (2)
Kepone	10.0	9.72	97 (5)	10.0	8.31	83 (4)
Bayer 29493	3.0	2.62	87 (4)	3.0	1.9	63 (4)
Bayer 22408	3.0	2.66	89 (4)	3.0	1.2	39 (4)
Ruelene	1.0	0.89	90 (4)	1.0	0.91	91 (4)
DDT	10.0	7.69	77 (3)	10.0	6.67	67 (3)
Dieldrin	10.0	6.59	66 (3)	10.0	7.87	79 (3)
Co-Ral	3.0	2.69	90 (4)	—	—	—

[a] Number of analyses are indicated in parenthesis.

extraction of milk and chromatography on Florex have been used for heptachlor and its epoxide (Johnsen *et al.* 1960). The carbamate, Sevin® (1-naphthyl *N*-methylcarbamate), has been recovered from milk with a 3:1 mixture of *n*-hexane and methylene chloride (Roberts *et al.* 1960).

The recovery of several organophosphate, chlorinated hydrocarbon, and carbamate insecticides from milk using two extraction techniques and the *in vitro* stability of certain organophosphates in milk are reported in this paper.

PROCEDURE.—*Extraction Techniques.*—Two extraction procedures were used to determine their relative efficiency for removing known amounts of certain insecticides (tables 1 and 2) from milk based on radiometric and colorimetric analyses. In Method A, the primary extraction solvents were acetonitrile and chloroform. A known amount of each insecticide in 0.1 ml. of acetone was added to a beaker containing 100 g. of fresh raw milk or whole homogenized milk; the contents were mixed thoroughly and transferred to a 500-ml. separatory funnel. One hundred and fifty ml. of acetonitrile was added to the separatory funnel and the mixture was shaken vigorously. The milk solids were precipitated and the supernatant liquid was filtered through a pledget of glass wool into another 500-ml. separatory funnel. The milk solids were washed with 150 ml. of chloroform, which was filtered through the pledget of glass wool and added to the acetonitrile extract. The acetonitrile-solubles, which included the water from the milk, and the chloroform-solubles were mixed thoroughly and then allowed to stand for several minutes for separation of the water layer from the organic solvent layer. The acetonitrile and chloroform mixture (bottom layer) were transferred to a 500-ml. Erlenmeyer flask, and the water layer was extracted again with a mixture of 35 ml. of acetonitrile and 35 ml. of chloroform. The combined acetonitrile and chloroform extracts were dried over anhydrous sodium sulfate and the solvent was removed by heating on a steam bath. Evaporation was aided by a gentle stream of air. The oily residue was transferred quantitatively with 25 ml. of acetonitrile to a 60-ml. separatory funnel. The acetonitrile was extracted three times with an equal volume of *n*-hexane to remove fats and phospholipids.

Aliquots of the acetonitrile, *n*-hexane, water, and milk residue were assayed for total radioactivity. The P^{32}-labeled Co-Ral, Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate), Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), and Bayer 22408 (*O*-naphthalimido-*O,O*-diethyl phosphorothioate) were assayed with an end window Geiger-Muller tube; the analysis of C^{14}-labeled DDT and Cl^{35}-labeled Kepone® (decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta [cd] pentalen-2-one), and dieldrin were made with a windowless gas-flow counter according to radiometric procedures previously described (Boyd & Arthur 1960). The percentage recovery of these insecticides from milk was based on the amount of radioactivity appearing in the final acetonitrile fraction from four separate determinations.

In recovery experiments with Sevin aliquots corresponding to 0, 5, 15, 25, 40, and 60 μgm. of Sevin were added to 100 g. of milk and then extracted with acetonitrile-chloroform as previously described. Colorimetric determinations of Sevin were made according to methods described by Whitehurst (1959). After the *n*-hexane-acetonitrile cleanup procedure, one drop of Carbowax 200 was added to the acetonitrile fraction and the acetonitrile was removed by evaporation on a steam bath. Two ml. of 0.1 N potassium hydroxide was added to the residue and allowed to stand for 5 minutes. The sample was then neutralized with 7 ml. of glacial acetic acid. After the addition of 1 ml. of color reagent (*p*-nitrobenzenediazonium fluoroborate), the colored solution was transferred to a 10-mm. cell and the optical density was read on a Beckman DU spectrophotometer at 475 mμ. Analyses for the recovery of 1-naphthol, a degradation product of Sevin, were performed in the same manner as described for Sevin except the potassium hydroxide hydrolysis step was omitted (Whitehurst 1959). Results on the recovery of Sevin and 1-naphthol were averaged from five determinations.

In Method B, 200 g. of whole milk fortified with the appropriate amount of insecticide was added to a 500-ml. separatory funnel and extracted three times with 250-ml. portions of diethyl ether and *n*-hexane (2:1 mixture). The diethyl ether and *n*-hexane extracts were combined, dried over anhydrous sodium sulfate, and filtered through a pledget of glass wool into a 1000-ml. Erlenmeyer flask. The solvents were evaporated on a steam bath and the resulting residue was transferred quantitatively to a 125-ml. separatory funnel with 50 ml. of acetonitrile. The acetonitrile was partitioned three times with an equal volume of *n*-hexane. Aliquots of the acetonitrile, *n*-hexane, and milk residue were assayed for total radioactivity, or the entire acetonitrile fraction was analyzed colorimetrically for Sevin as described for Method A. Again, the percentage recoveries were based on the relative amount of the

Table 3.—Recovery of insecticides from milk held under refrigeration at 12° C.

Days Incubation	Per Cent Recovery in Acetonitrile			
	Co-Ral	Ruelene	Bayer 22408	Bayer 29493
0	86	90	93	86
1	84	91	93	78
3	91	89	88	91
5	90	94	—	93
7	70	94	87	84
10	71	91	95	85
14	35	85	91	91

insecticide that appeared in the final acetonitrile fraction.

In Vitro Milk Studies.—The possibility that insecticides in milk would undergo activation or degradation as a result of enzymatic or bacterial action was considered *in vitro*. One hundred g. of fresh raw milk was placed in each of 12 cardboard cartons and the p.p.m. insecticide indicated in table 3 was added. The milk samples were held under refrigeration at 12° C. for 0, 1, 3, 7, 10, and 14 days, and at each time interval, two cartons of milk were extracted using the acetonitrile procedure (Method A) and analyzed radiometrically. An increase of radioactivity in the water or milk residue would indicate hydrolysis *in vitro*. To determine whether the Co-Ral, Bayer 22408, or Bayer 29493 were converted from the phosphorothioate to the phosphate structure, the acetonitrile-solubles were co-chromatographed on Celite with nonradioactive parent material and its corresponding oxygen analog (Boyd & Arthur 1960). The acetonitrile-solubles recovered from Ruelene fortified milk were identified by paper chromatography, using the acetonitrile and water (85:15) solvent system of Kaplanis *et al.* (1959).

Results.—The recovery of 5, 15, 25, 40, and 60 μgm. of Sevin from whole homogenized milk using the acetonitrile-chloroform extraction procedure (Method A) and colorimetric determination ranged from 93% to 98% with an average recovery of 95%; 1-naphthol recoveries ranged from 93% to 103% with an average of 97% (table 1). The sensitivity of the analytical method was approximately 0.025 p.p.m. Interference from closely related materials naturally occurring in milk that absorbed at 475 mμ. ranged below 0.025 p.p.m.

The recoveries of several insecticides from milk using the acetonitrile-chloroform or the diethyl ether-*n*-hexane extraction procedures are listed in table 2. The acetonitrile-chloroform extraction procedure was quite efficient for removing the organophosphates, Co-Ral, Ruelene, Bayer 29493, and Bayer 22408 from milk. Recoveries of Bayer 29493 and Bayer 22408 by the diethyl ether-*n*-hexane extraction procedure were considerably lower than recoveries using the acetonitrile-chloroform procedure. In partition distribution studies, more than 95% of the organophosphates as well as Sevin and Kepone remained in acetonitrile when partitioned with an equal volume of *n*-hexane.

Neither extraction procedure was satisfactory for removing DDT or dieldrin from milk. Acetonitrile-chloroform as the primary extraction solvents were 99% efficient in removing DDT from milk but 22% of the radioactivity was lost in the *n*-hexane cleanup (one extraction) of the acetonitrile-solubles. In the absence of milk extracts, only 72% of C^{14}-labeled DDT remained in acetonitrile when partitioned with *n*-hexane; this same distribution ratio for dieldrin was 73% in the acetonitrile and 27% in *n*-hexane. Using the acetonitrile extraction procedure, 23% of the dieldrin remained in the milk residue and 12% of the total radioactivity was accounted for in the *n*-hexane cleanup of the acetonitrile-solubles. The acetonitrile-chloroform extraction procedure was excellent for recovering Sevin or Kepone from milk, but the diethyl ether-*n*-hexane procedure was somewhat less efficient.

In general, acetonitrile-chloroform as the primary extraction solvents were quite satisfactory for removing a wide range of insecticides from milk.

In vitro incubation of Co-Ral, Ruelene, Bayer 29493, and Bayer 22408 in fresh raw milk for a maximum of 14 days and extraction of the milk by the acetonitrile-chloroform procedure yielded the results given in table 3. Based on the low percentage recovery in the acetonitrile fraction, Co-Ral was not stable in milk after incubation for 5 days. The 7-, 10-, and 14-day milk samples soured, and this process probably influenced the stability of Co-Ral. The radioactivity not recovered in the acetonitrile fraction was not removed from the milk residue with acetonitrile or chloroform. There was no increase of radioactivity in the water fraction. Ruelene, Bayer 29493, and Bayer 22408 were stable in milk for periods up to 14 days incubation. Percentage recoveries at the various incubation time intervals approximated those at zero time; variations were probably the result of extraction techniques.

All of the radioactive materials recovered in the acetonitrile fraction were the parent compounds as determined by Celite partition or paper chromatography. There was no *in vitro* formation of the oxygen analog of the phosphorothioates in milk.

Discussion.—Acetonitrile and *n*-hexane partitioning has been used rather generally as a cleanup procedure (Gunther & Blinn 1955) for separating pesticides from plant waxes and pigments. This solvent pair was also efficient as a cleanup method prior to determining Diazinon (Blinn & Gunther 1955), Co-Ral (Krueger *et al.* 1959) and Sevin (Roberts *et al.* 1960) residues in milk. Acetonitrile-chloroform as the primary extraction solvents for milk have not been reported. As compared with the diethyl ether-*n*-hexane extraction procedure, the acetonitrile-chloroform procedure was the easier and more rapid method, requiring less volume of solvent and centrifugation was not necessary to separate emulsions. The acetonitrile method was applicable to fresh raw milk or to homogenized milk and this avoids the necessity of separating butterfat from milk. The acetonitrile method was effective for removing from milk a wide range of insecticides, such as Sevin, Kepone, and four organophosphates. With the minimum of laboratory glassware and apparatus required for the acetonitrile method, it is feasible that this method could have broad application in the routine analysis of milk for insecticide residues.

In the acetonitrile-*n*-hexane cleanup of milk samples, good recoveries in the acetonitrile fraction depend upon the relative solubility properties of the particular insecti-

cide in this solvent pair. The poor recovery of DDT from milk was attributed to this solubility property; however, acetonitrile-*n*-hexane cleanup of plant extracts for determining DDT residues was satisfactory (Anglin & McKinley 1960).

Recoveries of Sevin and 1-naphthol from milk using the acetonitrile-chloroform procedure were considerably better than recoveries obtained by substituting methylene chloride or acetone for acetonitrile as the primary extraction solvent. The substitution of acetone for acetonitrile resulted in 50% to 60% recovery of Sevin and 1-naphthol recoveries were even lower. Although good recoveries of 1-naphthol were obtained from milk (table 1), the color intensity decreased considerably when the samples were left overnight in large volumes of acetonitrile. Milk samples for Sevin analysis should be processed completely within the day.

The possibility that biologically unstable insecticides would undergo bacterial or enzymatic degradation *in vitro* and would present no residue problem by the time the milk was processed for market appears remote. The apparent hydrolysis of Co-Ral (table 3) may have been the result of protein binding (Krueger *et al.* 1959). As was demonstrated for malathion (Norris *et al.* 1958) and methoxychlor (Cluett *et al.* 1960), the stability of insecticides in milk would eliminate the need for freezing milk samples if the analysis was made during the first week after collection.

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Biology of *Physcus* Sp. (Eulophidae, Aphelininae) from Burma[1]

T. W. FISHER, *Department of Biological Control, University of California Citrus Experiment Station, Riverside*

ABSTRACT

The female of *Physcus* sp. from Burma is a solitary primary endoparasite and is apparently host specific on *Aonidiella aurantii* (Mask.). The male develops as a solitary secondary parasite of *A. aurantii* through prepupae and young pupae of other endoparasites and its own species on which it develops as an ectoparasite. The sex ratio in the laboratory is 1 male to 10 females. Multiple oviposition in a single host may be requisite for reproduction. Continuous culture of *Physcus* sp. was achieved by a rotation system which assured the presence of suitable hosts for development of male parasites. Field evaluation is in progress.

Of the few published biologies which concern species of *Physcus*, one of the earliest and most complete was that by Taylor (1935, pp. 43–48) who observed that *P. varicornis* How. acted as a parasite of its own species. He recognized further that only the males of *P. varicornis* developed as secondary parasites of *Aspidiotus destructor* Sign. This characteristic was verified in other species of Aphelinidae by Flanders (1936) who determined that this

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relationship was a result of unmated females depositing their exclusively male-producing eggs on immature primary parasites of their own or related species. This contribution constituted a major break-through toward understanding the biologies of an important group of beneficial insects.

The present report concerns an undescribed species of *Physcus* which was found in 1957 by Dr. Paul DeBach of this department during foreign exploration for new parasites of the California red scale, *Aonidiella aurantii* (Mask.), in Burma. In its native habitat *Physcus* sp. was reportedly the dominant endoparasite of low density populations of *A. aurantii*, and several dozen females were shipped as immatures within their scale hosts to the quarantine insectary at Riverside, California. Mr. Harold Compere made the determination, and a description of this parasite, giving "*debachi*" as the specific name by which it is known colloquially in this department, is scheduled for publication in 1962 in the Jour. Ent. Soc. So. Africa (H. Compere, and D. Annecke).

Related species, which were introduced previously by others from different areas, include *Physcus flaviceps* Girault and Dodd (Flanders 1952), which is established on *Lepidosaphes beckii* (Newm.) in certain citrus areas of southern California, and *P. testaceus* Masi, which did not become established on fig scale in California. The purpose of this preliminary study was to develop information basic to development of a mass-production program.

GENERALIZED BIOLOGY.—The female *Physcus* sp. is a solitary primary endoparasite and is apparently host specific on *Aonidiella aurantii*. The male develops only as a solitary secondary parasite of *A. aurantii* through other endoparasites and its own species on which it develops as an ectoparasite. The species is arrhenotokous and the sex ratio in laboratory cultures is approximately 1 male to 10 females.

Mated Female Behavior and Female Development.—Scales attacked by mated females yield only female parasites. Preferred host stages are females in the second molt, third instar, or gravid state. Host feeding occurs mainly at the expense of prepupal male scales. No mated females were observed trying to oviposit in parasitized hosts. Tests showed that no reproduction occurred on purple scale.

Daily transfers involving several mated females individually isolated under $\frac{1}{2} \times 1$-inch glass cells with cloth tops revealed that at 80° F. the average number of eggs deposited was 11 and that 50% of a female's egg supply would be deposited by the third day. Oviposition time varied from 3 to 11 minutes. Ovipositing females lived an average life of 14 days at 80° F. Nonovipositing but mated females held at 60° F. with brief periodic removal for feeding and counting showed 12% mortality in 32 days.

Although space requirements were not worked out in detail, the results of two replications indicated that production from 12 females in a 5-inch tall jar, housing two scale-infested lemons, was three times that from 12 females in a $2\frac{1}{2}$-inch tall jar, housing one scale-infested lemon.

Contrary to expected results, this parasite displayed considerable tolerance to field dust. In tests with scale-infested lemons liberally coated with dust there was no apparent effect on oviposition. Mortality to adult or emerging *Physcus* sp. caused by dust was not readily apparent. A contributing factor may be that the female typically emerges through a hole she chews in the dorsum of the host, and she emerges dry with wings fully expanded.

Development of the immature female *Physcus* sp. includes four instars, as indicated by stages of mandibular growth. The fourth instar possesses functional spiracles. At 80° F. development is completed in 23 to 25 days on second-molt or older female California red scale.

Unmated Female Behavior and Male Development.—Eggs from unmated *Physcus* sp. produce only male progeny. In the laboratory the suitable host range is restricted in that oviposition occurs mainly on young unpigmented pupae of the primary parasites, *Comperiella bifasciata* How. and *Habrolepis rouxi* Comp., and young female pupae or, preferably, prepupae of its own species. At 80° F. this stage is available for approximately 24 hours. Prepupae of its own males also serve as hosts for the male. The second male then becomes a tertiary parasite of the scale insect. *P. flaviceps* in purple scale is not an acceptable host, probably because the male of *flaviceps* develops as an endoparasite of a sister pupa.

Oviposition by unmated *Physcus* sp. is rather wasteful because as many as five eggs may be deposited at a single insertion of the ovipositor. They are not carefully placed on the pupa of the host parasite but may fall haphazardly anywhere in the dried-out scale. This distribution plus internecine action are no doubt largely the reasons why only one male *Physcus* develops per host. A few virgin females were successfully mated after 54 days' storage at 60° F. with periodic removal to room temperature for feeding. Twenty-six virgins were all successfully mated after similar treatment for 24 days.

At 80° F. the immature male goes through three larval instars as an ectoparasite of its host parasite in 18 to 21 days. The first instar has 5 pairs of spiracles and the third instar has 9 pairs.

The mature male larva can be mistaken for a primary parasite, particularly if its gut is full. Presence of the host parasites' meconia shows the true relationship in this case. Two techniques were used for examining developing male *Physcus:* (1) Scale containing prepupae or very young parasite pupae of suitable host species were carefully removed from the host plant, usually a lemon fruit, and carefully stuck to a thin line of honey in an 8-dram vial. Virgin *Physcus* sp. were allowed to oviposit and were then removed. Subsequent development of the male could be viewed by reflected or transmitted light through the vial. (2) The second viewing technique was resorted to primarily for photographic purposes, but it proved to be a useful supplement to the technique described above. Following oviposition by the female parasite the scale was removed and stuck, ventral surface up, in a drop of Duco cement on a microscope slide. Several scales were placed in two rows. After 20 minutes the glue hardened sufficiently so that dissection could begin without pulling the scale from the glue. With a pair of fine stiff needles the periphery of the venter of the scale was picked away. As a result, the ventral portion of the scale could then be removed intact, thus exposing the parasite-host larva and its attacker, the immature male of *Physcus* sp. This procedure was performed dry. Parasites so exposed could then be

held for the remainder of their development by maintaining a sufficiently high relative humidity with saturated NaCl solution.

Adult Male.—Male *Physcus* sp. are typically one-half to one-third the size of the female. They are nearly a uniform black color and have "banana type" antennae. Exact longevity tests were not made, but it was learned that males held 34 days at 60° F. and then removed to 80° F. successfully impregnated newly emerged virgins.

Encysting of Physcus Eggs.—An undetermined proportion of female eggs deposited by mated *Physcus* sp. became encysted within early gravid California red scale. In many instances no apparent damage results because viable first-instar female larvae have emerged from the brown cysts which are readily discernible *in vivo*. Examples of apparent phagocytosis were also seen where the first-instar *Physcus* larva was dead in the encysted egg. Cysts forming around eggs may indicate that *Aonidiella aurantii* in southern California is not a perfect host for this parasite. Perhaps a distinct strain of scale is involved. Multiple oviposition in a single host may be requisite for reproduction, as shown by van den Bosch & Dietrick (1959) with the parasite *Bathyplectes curculionis* (Thomson) and its host *Hypera brunneipennis* (Boh.) in southern California.

LABORATORY PROPAGATION.—An understanding of the biology of *Physcus* sp. with its secondary males points to a basic problem in the culture of this parasite, namely keeping males in the population. To assure a reservoir of males, three methods were used:

(1) Virgin *Comperiella bifasciata*, which produced only male progeny, were allowed a 24-hour oviposition period in California red scale; 16 to 18 days later, when the *Comperiella* were prepupae, virgin *Physcus* sp. were added; 18 to 21 days later male *Physcus* emerged and were added to the culture. Male *Comperiella* which completed their development were, of course, no contamination problem in the *Physcus* cultures.

(2) A number of fruit containing California red scale parasitized by mated *Physcus* were regularly removed after a 48-hour oviposition period and held approximately 16 days away from adult parasites. These fruit were then replaced in the culture where emerging virgins, prior to mating, would have the opportunity of depositing male-producing eggs on young sister *Physcus* pupae.

(3) California red scale containing young pupae of *Comperiella* or *Habrolepis* were placed in a vial for a 4-hour sting period by virgin female *Physcus*. A ratio of 1 virgin per 4 pupae was used.

In a cage approximately 30×16×18 inches, method No. 2 was applied as a rotation system with reasonably good results.

EVALUATION.—The specialized biology of *Physcus* sp. from Burma may present a serious deterrent to establishment in California. It is expected that Dr. DeBach and his assistants will subsequently report on results of field colonization of this newly introduced parasite, as well as improvements in its mass culture.

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Absorption and Metabolism of Bayer 22408 by Dairy Cows and Residues in the Milk[1]

J. R. BUTTRAM and B. W. ARTHUR

Department of Zoology-Entomology, Auburn University Agricultural Experiment Station, Auburn, Alabama

ABSTRACT

P^{32}-labeled Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate) was applied dermally as a 0.5% emulsion to two Holstein dairy cows. Detectable quantities of the intact insecticide were isolated from the milk the first 6 days after treatment. Bayer 22408 equivalents in the milk were about 10 times higher than the actual Bayer 22408. No oxygen analog of the parent compound was isolated from milk, but it was the predominant nonhydrolyzed product of the feces. The fecal metabolites were toxic to stable fly (*Stomoxys calcitrans* (L.)) larvae, but not to house fly (*Musca domestica* L.) larvae.

O,O-diethyl *O*-naphthalimido phosphorothioate (Bayer 22408) appeared promising for control of several insect pests of livestock (Geary 1960). This phosphorothioate has a broad spectrum of insecticidal effectiveness, and it is low in toxicity to mammals (Boyd & Arthur 1960). The selective toxicity of Bayer 22408 was attributed to its greater stability in insects as compared to mammals. Rats degraded Bayer 22408 to phosphoric acid derivatives, which were eliminated primarily in the urine, but some intact insecticide was isolated from the feces of rats treated orally or dermally (Boyd & Arthur 1960). The results with Bayer 22408 in the feces of mammals suggested a practical approach for control of livestock pests that breed in feces. The need for a highly effective insecticide for controlling insect pests of dairy cattle is apparent.

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but few insecticides meet the stringent toxicological and pharmacological requirements necessary for safe usage.

The research reported herein with Bayer 22408 applied dermally to dairy cows concerns the magnitude and nature of residues in the milk, persistence on the hair, excretory rates, and the toxicity of the fecal metabolites to larvae of the house fly (*Musca domestica* L.) and the stable fly (*Stomoxys calcitrans* (L.)).

PROCEDURE.—*Materials and Chromatographic Analyses.*—P^{32}-labeled Bayer 22408 (1.7 mc./g.) was provided for this study through the cooperation of R. J. Geary, Vero Beach Laboratories, Inc., and chemists of the Farbenfabriken Bayer Company. The radiolabeled Bayer 22408 was greater than 98% pure as determined by Celite partition and paper chromatography, infrared analysis, and melting point.

The mobile solvent used in the Celite partition chromatography of Bayer 22408 and its oxygen analog (coded as Bayer 25820), and in the co-chromatography of these nonradioactive materials with the radioactive acetonitrile-soluble materials of milk, was the acetonitrile, *n*-hexane, and chloroform system described by Boyd and Arthur (1960). Water-soluble hydrolytic products from Bayer 22408 degradation were co-chromatographed with nonradioactive *O,O*-diethyl phosphoric and *O,O*-diethyl phosphorothioic acids on an anion exchange resin, Dowex 1×8 (Plapp & Casida 1958).

Bayer 22408 and Bayer 25820 were also separated by paper chromatography using 1-inch strips of Whatman No. 1 filter paper coated with 10% Dow Silicone 550 and a mobile solvent of acetone, acetonitrile, and water in a 14:1:20 (v/v) ratio (Gatterdam 1960). Most paper chromatograms were counted with an end window Geiger-Muller tube by the cm.-strip technique, but radioautograms were made of certain papers. The nonradioactive Bayer 22408 and 25820 were detected on papers by their fluorescence when exposed to ultraviolet light (3660 Angstrom). The hydrolytic products were separated on untreated paper strips using acetonitrile and water (85:15) as the mobile solvent (Kaplanis *et al.* 1959a).

Handling and Treatment of Cows.—Two Holstein dairy cows, designated in this paper as Cow A and Cow B weighing 940 pounds and 725 pounds, respectively, were housed in 12×12-foot stalls provided with overhead shelter. The cows were not exposed to rain or direct sunlight. Water and peanut hay were provided at all times, and during the milking period the cows were fed a 16% dairy feed concentrate. Milk production during the 15-day experimental period was 12.6 pounds per day for cow A and 20 pounds per day for cow B. Several days prior to treatment, teat dialators (Anson's Plastic Teat Tube) were inserted into the teats of cow A in an attempt to prevent external contamination of the milk samples. Milk was collected through plastic tubes in closed containers. Cow B was milked entirely by hand. The cows were milked twice daily and the udders were washed thoroughly with warm water prior to milking.

Bayer 22408 (3.0 g. of P^{32} plus 1.73 g. of the nonradioactive compound) was emulsified with 28.4 g. of xylene and 4.4 g. of Triton X-100 per quart of water. Each cow received 1 quart of this 0.5% emulsion, applied with a sponge to back and sides and with extreme care to prevent contamination of the udder. Application was made slowly to prevent runoff. No provision was made to prevent the cows from licking themselves.

Milk Samples.—Two pint cartons each of morning and evening milk from each cow each day were stored under refrigeration. Analyses were made within 24 hours after collection.

To determine total p.p.m. of apparent Bayer 22408, 1-ml. aliquots from each of the eight daily milk samples were evaporated to dryness in stainless steel planchets and radioassayed. The cumulative percentage of the applied dose eliminated in the milk was derived from this total p.p.m. of P^{32} in terms of the daily production of each cow.

Milk samples were fractionated with acetonitrile-chloroform as described by Timmerman *et al.* (1960a). Morning and evening samples from each cow were combined for analysis except during the first 3 days after treatment. Aliquots of the acetonitrile, *n*-hexane, water, and precipitated proteins were assayed for total radioactivity.

Classification of the acetonitrile-soluble P^{32} materials as Bayer 22408 or its oxygen analog may be misleading in interpretation of insecticide residue data. Therefore, the acetonitrile-soluble radioactive materials of the milk from each cow at daily intervals for 6 days posttreatment were co-chromatographed with carrier materials to determine the p.p.m. of Bayer 22408, the oxygen analog, or other metabolites.

Hair Samples.—To determine the residual effectiveness of Bayer 22408 and the chemical nature of the radioactive materials on the hair of the cows, hair samples were collected and processed as follows: Four hair samples (approximately 100 mg. each) were clipped from the sides of each cow (two samples from each side) at 0, 1, 3, 5, 7, 10, 14, and 17 days after treatment. Each hair sample was placed in 25 ml. of chloroform for about 12 hours. The hair residue was washed with another 25 ml. of chloroform and this extract was added to the first. The radioactivity in the chloroform was used to calculate the p.p.m. of Bayer 22408 on the hair. The p.p.m. of P^{32} materials remaining on the hair residue was also calculated. An aliquot of the chloroform solubles was partitioned with water, and the change in partition coefficient was compared with a predetermined partition coefficient of Bayer 22408 between this solvent pair.

An aliquot of the chloroform-soluble materials from the hair was chromatographed on paper (acetone, acetonitrile, and water) to determine the nature of P^{32} materials remaining on the hair. Duplicate paper chromatograms were developed on the chloroform solubles from the hair of each cow at 0, 1, 3, 5, 7, 10, 14, and 17 days after treatment. Hair samples collected at 7, 10, 14, and 17 days after treatment were also co-chromatographed on Celite with carrier Bayer 22408 and Bayer 25820.

Feces and Urine Samples.—The total feces from each cow was collected and weighed twice daily for the first 7 days after treatment. For the remainder of the experimental period, the feces was collected and weighed once each day. At each feces collection time, about 200 g. of a composite sample was placed in cardboard cartons and brought to the laboratory for analysis. The total p.p.m. of P^{32} materials was calculated from the radioactivity in duplicate 500-mg. samples of the wet feces. The cumula-

tive percentage of the applied P^{32} that was eliminated in the feces was also calculated.

A 100-g. sample of feces from each cow was collected at 6 hours and at 1, 2, 3, 5, 7, and 9 days after treatment. Each sample was extracted with 200 ml. water (12 hours) then with 200 ml. acetone (4 hours) (Dorough *et al.* 1960). The acetone and water extracts were combined and extracted twice with an equal volume of chloroform. The residue of the chloroform-soluble materials was partitioned between 25 ml. of acetonitrile and 25 ml. of *n*-hexane. The total P^{32} in each of these fractions and in the "unextractable" materials of the feces residue was determined. Characterization of the fecal metabolites was based on anion exchange and Celite partition chromatography.

The recovery of known amounts of Bayer 22408 added to control feces was determined by using the fractionation procedure as previously described.

One urine sample was collected during the experimental period from cow A on the second day after treatment. This sample was fractionated in a similar manner as described for the feces. The water-soluble hydrolytic products were chromatographed on paper strips and on anion exchange columns. The acetonitrile solubles were subjected to paper chromatography.

Bioassay of the Feces.—Duplicate fecal samples (about 200 g.) were collected daily from each cow for 5 days before treatment and for 21 days after treatment. Each sample was placed in a pint cardboard carton and stored in the laboratory at 27° C. Of the duplicate samples, 50 three-day-old house fly larvae were placed in one sample and 50 four-day-old stable fly larvae were placed into the other sample. From 2 to 3 ml. of water was added to each fecal sample daily. The toxicity of the Bayer 22408 or its metabolites to the fly larvae was based on the percentage of adult fly emergence from the feces before and after treatment of the cows.

RESULTS.—*Residues in Milk.*—The p.p.m. of Bayer 22408 and Bayer 22408 equivalents in milk from the two cows are given in table 1. Some P^{32} appeared in the milk at 6 hours after treatment. The radioactivity reached a peak in the milk at 5 to 6 days after treatment, then declined gradually. By 2 weeks after treatment, the radioactivity in the milk was below detectable levels (0.04 p.p.m.). This lower limit of detectability appeared somewhat high, since only 1-ml. milk samples were analyzed for total Bayer 22408 equivalents.

The higher concentrations of acetonitrile-soluble radioactive materials were at 1 and 4 days after treatment (table 1). The acetonitrile-soluble P^{32} was near the lower limit of detectability (0.01 p.p.m.) 7 days after treatment, and none was detectable 10 days after treatment. Residues based on actual Bayer 22408 were somewhat lower than when based on acetonitrile-soluble radioactivity. The oxygen analog of Bayer 22408 was not detectable in the milk of either cow. The P^{32}, which did not chromatograph as Bayer 22408, was eluted from Celite in the methanol fraction. The methanol fraction constituted 11% to 39% of the acetonitrile-soluble radioactive materials from the milk of cow A and 10% to 55% from the milk of cow B.

The nature of the metabolite(s) in the methanol fraction was studied by partition distribution of the radioactivity between chloroform and water and anticholinesterase activity as compared to Bayer 22408 and the oxygen analog. The chloroform and water partition coefficient of P^{32} Bayer 22408 was >100; nonradioactive Bayer 25820 was >100; and the P^{32} of the methanol fraction was 1.07. The molar concentration required to inhibit 50% of the cholinesterase of 30% human blood (Hensel *et al.* 1954) was 1.02×10^{-4} for Bayer 22408 and 3.6×10^{-8} for Bayer 25820; the Bayer 22408 equivalents of the methanol fraction from the milk was 1.47×10^{-5}. The anticholinesterase activity of the methanol fraction from Celite columns of the milk was not the result of oxygen analog eluted in this fraction, since carrier oxygen analog was not added to the columns and none was present in the milk *in vivo*.

Table 1.—Bayer 22408 and metabolites secreted in the milk of two dairy cows treated dermally with 1 quart of a 0.5% emulsion per cow.

Days After Treatment	P.P.M. Bayer 22408 in Milk Based on: Total P^{32} Materials[a]	Acetonitrile-Soluble Radioactivity[b]	Actual Bayer 22408[c]
0.25	0.086	0.062	0.053
1	.241	.094	.074
2	.364	.057	.044
3	.365	.039	.023
4	.526	.063	.046
5	.613	.036	.026
6	.522	.036	.023
7	.376	.017	<.010
8	.240	—	—
9	.211	—	—
10	.133	<.010	—
12	.079	—	—
14	<.040	—	—

[a] Averaged from eight 1-ml. aliquots of milk from each cow each day.
[b] Averaged from the acetonitrile-soluble radioactivity from duplicate 100 g. of milk from each cow.
[c] P^{32} Bayer 22408 characterized by co-chromatography on Celite with nonradioactive Bayer 22408.

The cumulative percentage of the Bayer 22408 applied to each cow that was eliminated in the milk is shown in figure 1. Less than 1% of the applied material was eliminated in the milk during the first 15 days after treatment.

Persistence of Bayer 22408 on the Hair.—The hair of each of the two cows contained about 18,000 p.p.m. of Bayer 22408 immediately after treatment. This residue declined to about 3500 p.p.m. by 17 days after treatment (fig. 2). The amount of Bayer 22408 on the hair of either cow was quite variable, probably a result of hair sampling and application techniques.

There was no oxidation or degradation of Bayer 22408 on the hair as determined by chloroform and water partition coefficients, Celite partition chromatography, and radioautographs of paper chromatograms. The Rf value of the radioactivity on the hair was the same as Bayer 22408 (Rf .115), and the radioactivity had the same chromatographic behavior on Celite as nonradioactive Bayer 22408. From 98% to 100% of the radioactivity partitioned from water into chloroform, which was the same partition distribution observed for known Bayer 22408. The two chloroform extractions of the hair samples were 95% to 99% effective in removing the radioactivity from the hair.

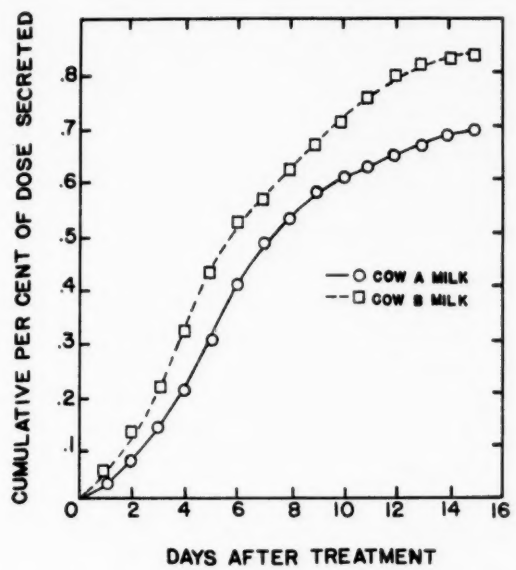

FIG. 1.—Secretion of Bayer 22408 and metabolites in the milk of cows following dermal treatment with 1 quart of 0.5% emulsion.

Excretion in Feces and Urine.—The highest concentration of P^{32} materials appeared in the feces 5 to 7 days after treatment (fig. 3). The cumulative percentage of the applied P^{32} that was accounted for in the feces was 32% for cow A and 40% for cow B (fig. 4). The P^{32} materials in the feces were separated into acetonitrile solubles, *n*-hexane solubles, water solubles, and "unextractable" materials of the fecal residue (table 2). Most of the acetonitrile solubles appeared in the feces during the first 5 days after treatment and were identified as residual Bayer 22408, the oxygen analog, and a methanol fraction (table 3), with the oxygen analog being the predominant material. Bayer 22408 added to control fecal samples resulted in 76% recovery in the acetonitrile fraction.

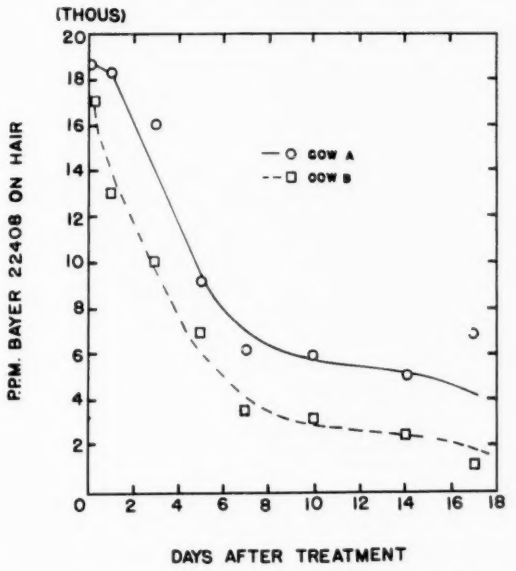

FIG. 2.—Disappearance of Bayer 22408 from the hair of dairy cows following application of a 0.5% emulsion.

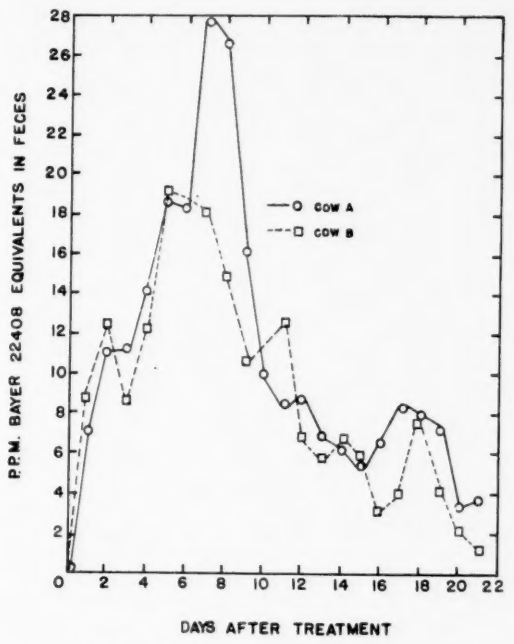

FIG. 3.—P.p.m. total Bayer 22408 equivalents in the feces of cows following dermal treatment.

The P^{32} of the methanol fraction from Celite columns of the organic solvent solubles of the feces (table 3) had a partition coefficient between chloroform and water of 0.66, slightly more polar than the P^{32} materials of the methanol fraction from milk. The constituents of the methanol fraction from feces were more potent anticholinesterase agents than the weak inhibitor of the milk. A molar concentration (based on Bayer 22408 equivalents) of 5.87×10^{-6} resulted in 75% inhibition of cholinesterase.

Table 2.—Extraction of the feces from cows treated dermally with Bayer 22408.

Days After Treatment	Per Cent of P^{32} Present in			
	Acetonitrile	*n*-Hexane	Water	Feces Residue
0.25	22.0	5.0	64.8	8.2
1	16.4	.3	57.8	25.5
2	14.9	.3	63.8	21.0
3	15.5	.1	70.0	14.4
5	13.7	.5	71.8	14.0
7	.5	.1	92.2	7.2
9	.3	.0	95.4	4.3

Table 3.—Celite partition and anion exchange chromatography of the acetonitrile and water-soluble radioactive materials recovered from the feces of cows treated with Bayer 22408.

Days After Treatment	Per Cent Present as				
	Celite-Acetonitrile			Anion-Water	
	Bayer 22408	O-analog	MeOH	$(C_2H_5O)_2P(O)OH$	$(C_2H_5O)_2P(S)OH$
1	4.5	11.4	0.5	17.2	40.6
3	6.7	8.8	.1	26.9	43.0
5	5.3	8.2	.2	18.5	53.3

The water-soluble P^{32} materials in the feces constituted more than 90% of the total radioactivity by 9 days after treatment (table 2). These hydrolytic products were separated into diethyl phosphoric and diethyl phosphorothioic acids (table 3). Considerable variation occurred between the two cows with regard to the percentage of each acid formed.

The urine collected from cow A at 2 days after treatment contained 95% hydrolytic products as determined by chloroform and water partition coefficients. The acetonitrile solubles of the chloroform extracts were separated on paper into Bayer 22408 (14%) and the oxygen analog (86%), while the water solubles were separated on an anion exchange column into diethyl phosphoric acid (37%) and diethyl phosphorothioic acid (63%). Attempts to separate the components of the water solubles by paper chromatography were not successful.

Toxicity of the P^{32} Materials in Feces to House Fly and Stable Fly Larvae.—The P^{32} materials in the feces were not effective in reducing the number of house fly larvae developing to adults, except in the feces collected during the first 24 hours after treatment (table 4). The emergence of stable flies was reduced in the feces collected at 3 to 6 days after treatment.

DISCUSSION.—Bayer 22408 possesses several properties that are different from most organophosphates. The high insecticidal efficiency (Geary 1960) and low toxicity of Bayer 22408 to mammals (Boyd & Arthur 1960) place it among the safest of the organophosphates. Bayer 22408 was absorbed more readily through the skin of mammals than Co-Ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate) (Krueger *et al.* 1959, Kaplanis *et al.* 1959b, Robbins *et al.* 1959) or Delnav® (a mixture of 2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate and related compounds) (Plapp *et al.* 1960), and a large portion of the absorbed Bayer 22408 appeared intact in the feces of rats (Boyd & Arthur 1960), guinea pigs (Gatterdam 1960), and sheep (Timmerman *et al.* 1960b). The significance of this absorption and distribution pattern may lie in the partial control of livestock insects that breed in feces, as was suggested by Boyd and Arthur (1960) and demonstrated by the research reported in this paper.

There was a considerable variation in the p.p.m. of Bayer 22408 in the milk of the two cows. The larger cow (cow A) produced less milk than cow B, but the milk from cow A always contained more Bayer 22408 than the smaller cow. The milk from cow A was allowed to flow through teat dialators into closed containers to minimize the possibility of external contamination of the milk samples. It is conceivable that "pharmacological zero" residues in milk may be possible when small cows in high milk production are treated. However, treatment of large cows in low milk production may result in detectable pesticide residues.

Residues of insecticides based on total p.p.m. of radioactive materials or based on the organic solvent-soluble radioactive materials are often misleading, since organophosphates are degraded to inorganic phosphates, which are incorporated into normal phosphorus-containing metabolites that partition from an aqueous medium into an organic solvent. Characterization of the radioactive materials must be accomplished to establish the presence or absence of toxic residues. An assumption that all acetonitrile-soluble P^{32} materials were Bayer 22408 or the oxygen analog would have been misleading, although good recoveries of Bayer 22408 added to milk were obtained in the acetonitrile fraction (Timmerman *et al.* 1960a). The data in table 1 clearly illustrate this point.

Table 4.—Emergence of adult house flies and stable flies from larvae placed in feces of cows before and after treatment with Bayer 22408.

Days After Treatment	Per Cent Emergence	
	House Fly	Stable Fly
1	14	95
2	80	31
3	73	.5
4	88	15
5	78	1
6	56	0
7	83	37
8	57	50
10	100	92
12[a]	—	87
Control[b]	71	93

[a] No mortality of stable fly larvae in the 13- through 21-day fecal samples.
[b] Averaged from 4 days prior to treatment of cows.

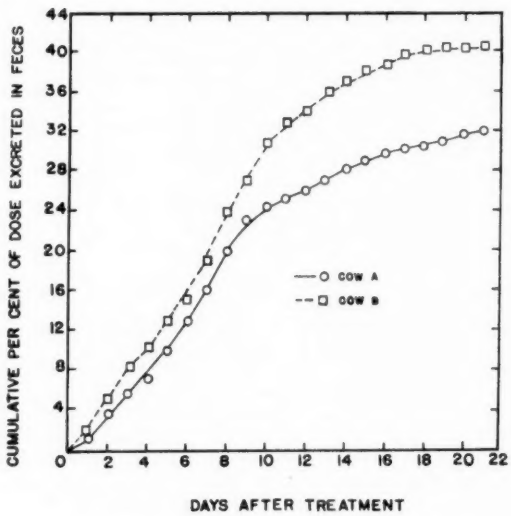

FIG. 4.—Cumulative percentages of the applied Bayer 22408 excreted in the feces of dairy cows.

The absence of the oxygen analog of Bayer 22408 in the milk of cows was most interesting, since large quantities of radioactive materials in the feces were characterized as the oxygen analog. Cows appeared quite successful in eliminating foreign materials from the body via the urine and feces, whereas the milk remained comparatively free of contamination. Less than 1% of the total radioactivity applied dermally appeared in the milk (fig. 1), while about 35% appeared in the feces (fig. 4).

Both cows became quite excited during the application of Bayer 22408, probably a result of the emulsion irritation to the skin. However, no signs of phosphate poisoning were observed throughout the test period.

The complexity of the metabolic scheme of Bayer 22408 in mammals has not been elucidated thoroughly. The occurrence of more diethyl phosphoric acid immediately after treatment than at subsequent time intervals (Boyd & Arthur 1960) was also observed in cows. This unique metabolic scheme needs more thorough investigation.

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The Toxicity of Various Fumigants to the Cadelle, *Tenebroides mauritanicus*[1]

E. J. BOND and H. A. U. MONRO[2]

ABSTRACT

The toxicity of 13 fumigants to *Tenebroides mauritanicus* (L.) (Coleoptera:Ostomidae) has been determined and the results have been compared with data on *Sitophilus granarius* (L.) and *Tribolium confusum* Jacquelin duVal. Most of the fumigants were less toxic to the cadelle than they were to the other species. Two of the fumigants, ethylene dibromide and ethylene chlorobromide, had a very pronounced delayed action on the cadelle; phosphine, a fumigant which was very toxic to the cadelle failed to give complete kill on *S. granarius* even when the concentration was increased 3-fold above the LD_{90} level.

The order of toxicity of different fumigants to different species of insects may be extremely variable. For instance the relative resistance of two species may change from one poison to another as is the case with methyl bromide and hydrogen cyanide against the cadelle, *Tenebroides mauritanicus* (L.) (Coleoptera:Ostomidae), and the granary weevil, *Sitophilus granarius* (L.). Methyl bromide is about four times as toxic to the granary weevil as to the cadelle while hydrogen cyanide is more toxic to the latter species. Because of this variability in living material the toxicity of each fumigant to a given species must be determined separately. This paper reports a study of the toxicity of several fumigants to the cadelle in comparison with that of two relatively well known species, the granary weevil and the confused flour beetle, *Tribolium confusum* Jacquelin duVal. Data are also given on the rate of action of these fumigants on the cadelle.

The cadelle was chosen for this investigation because it is more resistant to some fumigants than are other stored product insects. It is often found in association with these insects and thus may serve as an index for the success of treatments against them. The fourth instar larva was used in most tests because large numbers of this stage in a uniform state of development can be obtained readily and because it is one of the most resistant stages. However,

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the toxicity of methyl bromide to all other stages of the cadelle was also studied.

MATERIALS AND METHODS.—In handling the test insects precautions were taken to obtain uniform cultures and to fumigate them when they were in a normal metabolic condition.

The cadelle was reared in 1-quart fruit jars according to methods previously described (Bond & Monro 1954). New cultures were started twice a week so that a continuous supply of uniform test insects was available. The fourth instar larvae, which were used for most tests, were taken when they were full grown and in the same state of development. They were exposed to the fumigant in special trays each holding 30 cells made of glass rings 1-inch I.D. and $\frac{3}{8}$ inch high, glued to a base made of a $\frac{1}{4}$-inch thick plastic sheet. These trays thus afforded individual compartments that kept each larva separate and prevented fighting; thus the disturbance factor which would affect the metabolic rate and hence the resistance of the insects to the fumigant was eliminated. The insects were removed from the food and placed in the dishes 16 hours before fumigation so that they would recover from the effects of handling before treatment.

The granary weevil was reared in 1-quart fruit jars on soft spring wheat. Two thousand mature adults were placed on 700 grams of wheat and allowed to lay eggs, then they were moved to a new jar of wheat every 2 days so that a continuous supply of insects uniform in age and development was maintained. The adult insects were used for test purposes 2 weeks after emergence from the grain. About 40 were exposed together in petri dishes 100×15 mm. which had a specially constructed plastic barrier on the rim that prevented the weevils from escaping, yet left them completely exposed to the gas. Ten grams of wheat was placed in each dish. These arrangements allowed the insects to feed and be exposed to the gas while in a natural undisturbed state.

The confused flour beetle was reared on whole wheat flour to which brewers' yeast had been added. One hundred and ninety grams of flour and 10 grams of brewers' yeast were placed into plastic dishes 5 in. square by $1\frac{1}{2}$ in. high to which 500 eggs were added to allow development of the insects to the adult stage. Adults were used in the fumigation tests when 1 to 2 weeks old. About 40 were placed in each petri dish without food the afternoon before treatment. A small amount of flour was added to the dishes the day after treatment to serve as food for the survivors.

The fumigants used in these tests, listed in table 2, were the technical grades which are normally used in commercial treatments.

EXPERIMENTAL PROCEDURE.—The insects were exposed to the fumigants in the equipment described by Monro & Buckland (1955). Six individual fumigation chambers allowed treatment of insects with six graduated concentrations of fumigant under otherwise identical conditions. Normally the insects were exposed to the fumigant for 5 hours at 25° C. and 70% relative humidity and then returned to a rearing room having the same temperature and humidity. The mortality was assessed 7 days after treatment; any insects that showed any movement when touched or when placed under the light of a 60-watt bulb were recorded as survivors. The results were corrected for natural mortality by Abbott's formula (Abbott 1925) and dosage response curves were drawn on logarithmic probability paper. These curves were tested for goodness of fit by the method of Litchfield & Wilcoxon (1949) and the LD_{50} and LD_{99} were determined from the curves.

RESULTS.—*Response of Various Stages of the Cadelle to Methyl Bromide.*—When cadelle eggs of different ages were exposed to methyl bromide the oldest eggs were found to be the most resistant, requiring 7 mg./l. for complete kill, while the youngest eggs were the least resistant, requiring only 4 mg./l. The per cent mortality of eggs of the different ages exposed to 4, 5, 6 and 7 mg./l. of methyl bromide were as follows:

Conc. CH_3Br (mg./l.)	Age (days)					
	1	2	3	4	5	6
4	100	49	32	88	50	52
5	—	100	88	100	100	75
6	—	—	100	—	—	87
7	—	—	—	—	—	100

Dosage figures at the 50% and 99% mortality levels for the larval stages, the pupae and adults are given in table 1. The relative resistance of the various stages at the LD_{99} level is pupa > adult > 4th larva > 3rd larva > 2nd larva > 1st larva > eggs.

Comparative Toxicity of Various Fumigants to the Cadelle.—The LD_{50} and LD_{99} for the various fumigants are given in table 2. Some of the data listed in this table give only the approximate dosage required because it was not always possible, in these experiments, to obtain the mortality curves from which exact figures could be extracted. The order of toxicity for the cadelle larva at the LD_{99} level is hydrogen phosphide > acrylonitrile > chloropicrin > hydrogen cyanide > sulphuryl fluoride > sulphur dioxide and methyl bromide > ethylene dibromide > ethylene oxide > ethylene chlorobromide > ethylene dichloride > carbon disulphide > carbon tetrachloride.

Different fumigants exhibit different types of action: Some affect the insect immediately; others have a delayed effect. The lethal effect of ethylene oxide, ethylene dichloride, chloropicrin, methyl bromide and acrylonitrile was almost complete within 48 hours after the exposure and insects which survived after this time usually developed to adults. Sulphur dioxide had a delayed killing

Table 1.—Dosages (mg./l.) required to kill 50% and 99% of various stages of the cadelle during a 5-hour exposure to methyl bromide.

Stage of Insect	Mortality Level	
	LD_{50}	LD_{99}
Egg		ca. 7
1st instar larva		ca. 13.5
2nd instar larva		ca. 14.5
3rd instar larva	12.5	16.7
4th instar larva	14.8	22.0
Pupa (3–5 days old)	17.6	24.2
Pupa (5–8 days old)		21.0
Adult (3–5 days old)	16.7	22.2
Adult (2–4 weeks old)	12.8	21.8

Table 2.—The concentration of fumigant (mg./l.) required to kill 50% and 99% of the insects when exposed for a 5-hour period.

Fumigant	Cadelle (4th larva)		Granary Weevil (adults)		Confused Flour Beetle (adults)	
	LD_{50}	LD_{99}	LD_{50}	LD_{99}	LD_{50}	LD_{99}
Acrylonitrile	4.0	8.0	1.3	2.2	3.0	3.9
Hydrogen cyanide	2.5	13.3	8.1	13.5	0.7	1.1
Chloropicrin	4.9	11.2	3.5	30.0	6.8	11.5
Methyl bromide	14.5	23.0	4.2	5.6	9.0	12.8
Ethylene dibromide	13.5	25.0	3.8	6.9	3.5	6.2
Ethylene chlorobromide	24.0	68.0	4.3	9.6	—	—
Ethylene dichloride	43.0[a]	72.0[a]	44.5[a]	92.5[a]	—	32
Ethylene oxide	18	35	5.2	7.2	18.3	25.5
Sulphur dioxide	14.3	23.5	5.2	10.2	4.8	7.2
Carbon disulphide	25.0[a]	34.5[a]	40.5	65.0	66	112
Carbon tetrachloride	100[a]	—	56.5[a]	97[a]	—	55[a]
Sulphuryl fluoride	11.3	16.3	1.6	3.5	10	11
Hydrogen phosphide	—	ca 1	ca 1	>13	—	ca 4

[a] Concentration given is for a 24-hour exposure period.

action; most of the victims succumbed within 24 hours after treatment but mortality at the rate of 1% per day continued to the seventh day. Delayed action was even more pronounced in larvae treated with ethylene dibromide and ethylene chlorobromide, where nearly all insects were still alive on the day after the exposure. Subsequently, however, they died at the rate of 12% per day for the next 5 days and 3% per day for the following 9 days. The mortality after 15 days was 92% for ethylene dibromide treated larvae and 89% for those treated with ethylene chlorobromide. None of these larvae pupated. Carbon disulphide had the opposite effect in that it anesthetized the insects immediately after treatment; most of the affected larvae were in a flaccid paralysis and were yellowish in color. Within 4 days they began to recover so that 80% responded to stimulation by the seventh day and 94% appeared normal 2 weeks after the treatment. Hydrogen cyanide produced a similar effect. All larvae exposed to concentrations above 2 mg./l. were completely paralyzed soon after exposure and remained in this state for more than 20 hours after treatment. Recovery then took place gradually, occurring first in insects treated at the lower doses and later in the others. It will be noted from figure 1 that most of the fumigants caused a decrease in viability of survivors so that some of them failed to reach the pupal stage.

Hydrogen phosphide was found to be extremely toxic to the fourth instar cadelle larva, a concentration of 1 mg./l. producing complete kill after a 5-hour exposure. The granary weevil on the other hand was considerably more resistant; 1 mg./l. for 5 hours killed 50% of the insects, 4 mg./l. killed 90% and all subsequent concentrations up to 13 mg./l. killed only 90% of the insects.

Discussion.—The results presented here show that most of the fumigants tested were less toxic to the cadelle than they were to the granary weevil or the confused flour

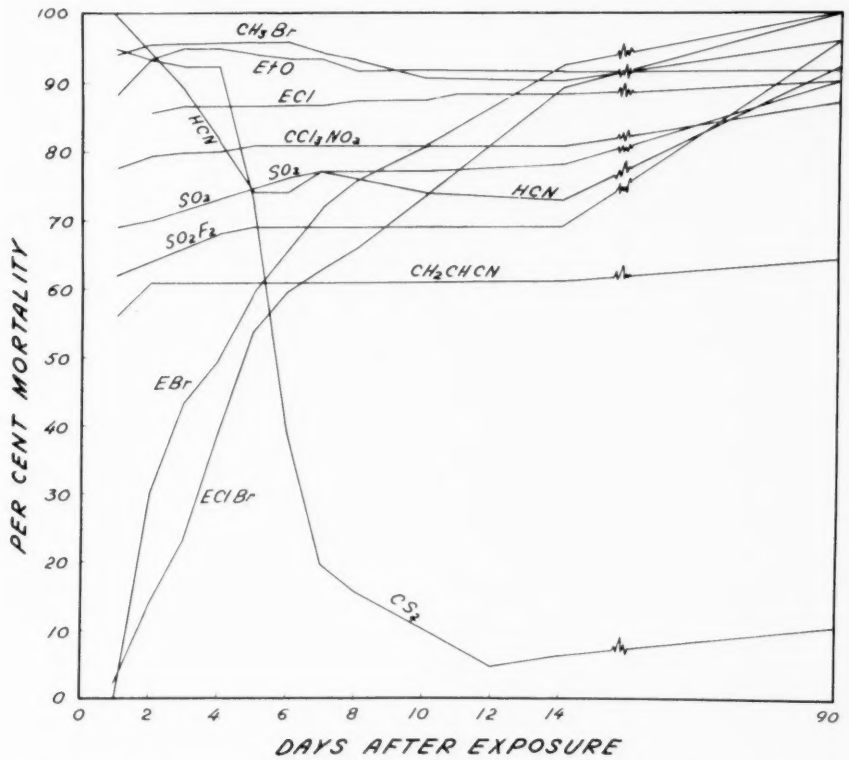

Fig. 1.—The mortality of cadelle larvae at various intervals after exposure to various fumigants (EtO-ethylene oxide, E Cl-ethylene dichloride, E Br-ethylene dibromide, E Cl Br-ethylene chlorobromide).

beetle. Previous results have shown that the toxicity of at least one fumigant (methyl bromide) to the cadelle is related to respiratory rate (Bond 1956). By comparing fumigant toxicity with the respiratory rates of the insect species used in these experiments[3] it was found that the cadelle, the insect with the lowest respiratory rate, was more resistant than the other insects to acrylonitrile, methyl bromide, ethylene dichloride, ethylene chlorobromide, ethylene oxide, sulphur dioxide, carbon disulphide, carbon tetrachloride and sulphuryl fluoride. This finding suggested that respiratory rate, which may well influence fumigant uptake, was related to the toxicity of these fumigants. However, the fumigants, ethylene dichloride, chloropicrin and hydrogen phosphide did not show this relationship in that they were more toxic to the cadelle than to the other insects. A comparison of the toxicity of the various fumigants to the respiratory rate of the granary weevil and the confused flour beetle did not show any marked relationship.

It is noteworthy that hydrogen cyanide, which is a respiratory poison, was more toxic to the cadelle than to the granary weevil, whereas methyl bromide, a fumigant which does not readily affect insect respiration, was four times more toxic to the granary weevil. The confused flour beetle which is intermediate between these two insects in respiratory rate and in resistance to methyl bromide was extremely susceptible to hydrogen cyanide. Hydrogen phosphide, like hydrogen cyanide, produced paralysis in all the insects tested and was much more toxic to the cadelle than to the granary weevil. The toxicity curve for hydrogen phosphide against the granary weevil had a gradual slope so that much greater concentrations were required to give complete kill than were needed to produce 90% mortality. The survival of some granary weevil after treatment with high doses suggested that either these insects did not absorb correspondingly greater amounts of the fumigant as the surrounding concentration was increased or that greater amounts were required at the site of action to produce a lethal effect. Such tolerance as these surviving insects exhibited provides conditions that favor the development of resistant populations in commercial fumigation practices. Therefore, the survivors of high dosage treatments are being bred through to determine the rate at which resistant populations may develop, and the rate of uptake of hydrogen phosphide by this insect is being investigated to determine the toxicity of the poison in terms of the amount absorbed.

Most fumigants exert their effects on insects soon after treatment; however, some produce a rapid paralysis from which the insects eventually recover and others act very slowly so that their effects may continue for days or weeks after treatment. Carbon disulphide, hydrogen cyanide and hydrogen phosphide produced rapid paralysis at relatively low concentrations so that many insects recovered and resumed normal activity. Ethylene dibromide and ethylene chlorobromide, both fumigants with low boiling points and high sorption values, continued to exert their effects on the insects for several weeks after treatment. It is interesting to note that ethylene dichloride did not have a delayed effect on the cadelle but on the confused flour beetle its delayed action continued for 20 days after treatment.

The results presented here show that different poisons act differently on different species of insects both in their toxic effects and in the rate at which they exert these effects. Because such effects as paralysis with subsequent recovery and delayed mortality may occur, the assessment of mortality and subsequently insecticidal effectiveness should be based on some knowledge of the response of the insect to the poison and the rate of action of the poison on each particular species concerned.

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[3] Rate of oxygen consumption:

4th instar cadelle larvae 786.3 ± 39.3 μl. gm. hr.
Confused flour beetle adults 1082 ± 17.3 μl. gm. hr.
granary weevil adults 1445.9 ± 35.9 μl. gm. hr.

Residues on Oranges Resulting from the Use of DDT, Parathion, Phosdrin, and TDE for the Control of Orangeworms[1]

E. L. Atkins, Jr., R. C. Blinn, T. R. Fukuto, and F. A. Gunther[2]
University of California Citrus Experiment Station, Riverside

Abstract

The residual behavior of DDT, parathion, Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate), and TDE on oranges following treatment designed for the control of various orangeworms has been studied. It was determined that a 2-week waiting period between treatment and harvest will result in residues well below the existing tolerance levels.

The usefulness of DDT, parathion, Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate), and TDE for the control of various orangeworms including orange tortrix, *Argyrotaenia citrana* (Fern.), fruit-tree leaf roller, *Archips argyrospilus* (Wlkr.), citrus cutworm, *Xylomyges curialis* Grote, variegated cutworm, *Peridroma saucia* (Hbn.), pink scavenger caterpillar, *Pyroderces rileyi* (Walsm.), and the western tussock moth, *Hemerocampa vetusta* (Bdv.), has been reported (Atkins 1951, Atkins 1958a, Atkins 1958b, Carman *et al.* 1959). Although residue data for some of these insecticides on citrus have been published (Carman *et al.* 1950), they were from higher dosages designed for control of several scale insects. It is the purpose of this paper to present residue data for DDT, parathion, Phosdrin, and TDE on Valencia oranges from dosages designed for the control of the above-named orangeworms, and to establish the suitability of a 14-day waiting period between treatment and harvest.

Materials and Methods.—Mature Valencia orange trees were treated on November 14, 1957, with 1, 2 or 4 pounds of a wettable powder formulation of 50% DDT or TDE per 100 gallons of water at the rate of 2.5, 5, or 10 pounds of compound per acre; or with 0.5, 1, or 2 pounds of a wettable powder formulation of 25% parathion per 100 gallons of water at the rate of 1, 2, or 4 pounds of parathion per acre. Boom-spray applications were used to achieve thorough distribution of the spray mixtures to outside or peripheral parts of the trees. On May 20, 1957, mature Valencia orange trees were treated with Phosdrin at the rate of 1, 1.5, or 2 pounds of technical grade Phosdrin per acre by means of a hand sprayer.

Mature orange fruit samples were collected for assay of residues 1, 3, 7, 14, 21 and 42 days after treatment. Four peripheral fruits (one from each quadrant) were picked from each of eight trees in each plot, and the resulting 32-fruit sample was processed as a unit. The three replicates for each treatment were processed separately.

Unwashed fruits were weighed, peeled, and the peel and pulp weighed separately before the peel was processed with *n*-hexane for the DDT and TDE samples and with benzene plus dilute hydrochloric acid for the parathion samples in the equipment and manner previously described (Gunther & Blinn 1955) to afford final stripping solutions. For Phosdrin assay, whole unwashed fruits were ground, mixed well, and aliquots blended in a Waring Blendor with chloroform.

Table 1.—Residues of DDT, parathion, and TDE as parts per million in the whole fruit,[a] on and in mature Valencia oranges.

Days After Treatment	DDT[b] (lb./a.)			Parathion[c] (lb./a.)			TDE[d] (lb./a.)		
	2.5	5	10	1	2	4	2.5	5	10
1	—	—	—	0.5	0.9	1.7	1.0	2.6	3.2
	—	—	—	0.5	0.5	2.0	1.0	2.1	2.9
	—	—	—	0.4	0.6	1.0	1.3	2.4	2.8
7	0.9	1.9	3.0	0.1	0.5	1.2	0.3	1.2	1.8
	1.0	2.0	3.4	0.2	0.7	1.0	0.4	0.9	2.7
	1.2	2.1	3.0	0.2	0.5	1.1	0.3	0.9	3.2
14	0.5	1.2	2.8	0.1	0.3	0.8	0.4	1.1	2.5
	0.6	1.3	3.0	0.1	0.4	0.7	0.8	1.2	2.6
	0.7	1.3	3.8	0.1	0.5	0.8	0.8	1.2	2.2
21	0.7	1.9	3.0	0.1	0.6	0.5	0.9	1.1	1.1
	0.7	1.7	2.8	0.2	0.3	0.3	0.4	1.4	2.1
	0.8	1.9	2.8	0.1	0.3	0.5	0.3	1.1	1.9
42	0.3	0.9	2.3	0.1	0.2	0.3	0.7	0.8	1.6
	0.4	1.0	2.6	0.1	0.2	0.4	0.5	0.8	1.5
	0.3	0.9	2.3	0.2	0.3	0.3	0.5	0.8	1.3

[a] These oranges had 24.2 weight-per cent peel from 180 measurements.
[b] All values corrected for background (0.4 p.p.m.) and for recovery (104%).
[c] All values corrected for background (<0.1 p.p.m.) and for recovery (85%).
[d] All values corrected for background (0.5 p.p.m.) and for recovery (100%).

Aliquots of these stripping solutions were analyzed for DDT and TDE by the combustion-chloride procedure (Gunther & Blinn 1955), for parathion by a modification (Gunther & Blinn 1955) of the Averell & Norris (1948) colorimetric procedure, and for Phosdrin by a cholinesterase inhibition procedure (Hensel *et al.* 1954).

Results.—Results of the residue analyses for DDT, parathion, and TDE on and in Valencia oranges are presented in table 1. Results for Phosdrin are given in table 2. All values are expressed as parts per million of the apparent insecticide based upon weight of whole fruit.

Discussion.—Calculation of degradation and persistence half-life values (Gunther & Blinn 1955) from this short experiment, in the usual manner, show that DDT has an approximate persistence RL_{50}[3] value of 50 days, while parathion and TDE have approximate degradation RL_{50} values of 15 and 23 days, respectively, and persistence RL_{50} values of at least 54 and 42 days, respectively. The RL_{50} for Phosdrin is about 2 days.

[1] Paper No. 1266, University of California Citrus Experiment Station, Riverside, California. Accepted for publication November 22, 1960.
[2] The authors wish to thank Mrs. L. M. Elliott and Miss P. A. Roberts for assistance in the laboratory.
[3] RL_{50} is a suggested replacement term for "half-life" in this usage and means "Residue Life" or "Residue Loss," 50%. The use of "half-life" should probably be reserved for the field of classical reaction kinetics. This new terminology was suggested by Dr. George C. Decker, and is hereby endorsed to avoid confusion with the former term which has been correctly used in the present technical sense but which was confused by some individuals who thought it had been preempted by radiochemists.

Table 2.—Residues of phosdrin as parts per million in the whole fruit,[a] on and in single samples of mature Valencia oranges.

DAYS AFTER TREATMENT	1 LB./ACRE	1.5 LB./ACRE	2 LB./ACRE
0[b]	0.07	0.15	0.16
1	0.02	0.04	0.05
3	0.01	0.04	0.03
7	Nil[c]	0.01	0.01
14	Nil[c]	Nil[c]	Nil[c]

[a] All values corrected for background (<0.01 p.p.m.) and for recovery (80%).
[b] Picked when fruit had dried.
[c] Nil means less than 0.01 p.p.m.

Since tolerances for DDT, parathion, and TDE on citrus fruits have been set at 7, 2, and 7 p.p.m., respectively, on a whole-fruit basis, the recommended 14-day waiting period after treatment before harvesting (Carman *et al.* 1959) will result in residues well below the tolerance levels.

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Magnitude and Nature of Residues in Tissues and Eggs of Poultry Receiving Ruelene in the Feed[1]

J. R. BUTTRAM and B. W. ARTHUR, *Department of Zoology-Entomology, Agricultural Experiment Station, Auburn University, Auburn, Alabama*

ABSTRACT

Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate) was rapidly metabolized by laying hens receiving the compound in the diet for 7 days at 100 p.p.m. Acetonitrile-soluble residues were dissipated from several tissues by the time the hens were returned to normal feed for 3 days, but appeared in egg yolks at 3 to 5 days after the hens were returned to normal feed. These acetonitrile-soluble residues were not characterized as Ruelene. Ruelene was degraded to phosphoric acid that was incorporated into normal phosphorus-containing constituents, particularly in bone tissue. Only 29% of the Ruelene consumed in the feed was eliminated in the feces during the 21-day experimental period. Six metabolites were isolated from the excreta and there was a possibility that nine metabolites were present. *O*-methyl phosphoric acid and 4-*tert*-butyl-2-chlorophenyl methyl phosphoric acid were tentatively identified. The *in vivo* degradation of Ruelene is complex, involving enzymatic attack at the three different groupings of the molecule.

Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate) possesses promising parasiticidal and systemic insecticidal activity in livestock (Shaver & Landram 1959, McGregor *et al.* 1959, Timmerman *et al.* 1960). The practical use of Ruelene depends not only upon its anthelmintic and insecticidal effectiveness but upon the magnitude and nature of residues deposited in edible tissues. The synthesis of Ruelene with a P^{32}-label by chemists of The Dow Chemical Company afforded an opportunity to study its metabolic fate and residue pattern in tissues and eggs of hens receiving the insecticide mixed in the feed.

PROCEDURE.—P^{32}-labeled Ruelene (1.35 g.; 0.69 mc./g.) was dissolved in 675 ml. of acetone and thoroughly mixed with 27 pounds of laying mash, yielding 100 p.p.m. Ruelene in the feed. The feed and acetone solution were mixed in a galvanized tub until the acetone evaporated and the radioactivity was evenly dispersed as determined by radioassaying several 100-mgm. samples with an end window Geiger-Müller tube.

The feed fortified with Ruelene was provided free-choice to 24 White Leghorn hens weighing approximately 1.55 kg. each. The hens were about 18 months old, and in 40% to 50% egg production. Birds were housed individually in wire cages and the droppings were collected on protectopaper placed underneath each cage. Feed containers were secured on the outside of the cages to minimize contamination of the feces with the radioactive feed wasted during feeding.

The hens received the feed containing Ruelene for a maximum of 7 days and then certain hens were returned to normal feed. Nine of the 24 hens were sacrificed in groups of three at 1, 3, and 7 days after being on radio-

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active feed. The remaining 15 hens were sacrificed at 1, 3, 7, 10, and 14 days after the return to normal feed. The liver, drumstick, breast, gizzard, kidneys, brain, and a portion of the skin, fat, and bone from each hen were wrapped in aluminum foil and stored in a deep freeze until analyzed for Ruelene or its metabolites.

Prior to sacrificing a hen, 5 ml. of blood was withdrawn from the heart region with a 10-cc. syringe containing a few drops of heparin. The blood samples were refrigerated until analyzed. Duplicate samples of the tissues of each hen were analyzed for total Ruelene equivalents using methods described by Dorough *et al.* (1961a). Tissues containing greater than 0.04 p.p.m. Ruelene equivalents were fractionated to determine the amount of acetonitrile-soluble residues. From 10 to 20 g. of all tissues were used with duplicate analysis per tissue, except for the kidney and brain where the entire tissue was analyzed. The fractionation procedure (Anderson *et al.* 1959) was the same as described for determining Co-Ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate) and metabolites in poultry tissues and eggs (Dorough *et al.* 1961a). This procedure consisted of acetone followed by benzene extraction of tissue homogenates, removal of the benzene and acetone on a steam bath, extraction of the aqueous residue with *n*-hexane, and finally, partitioning the *n*-hexane with an equal volume of acetonitrile. The p.p.m. Ruelene or metabolites in the acetonitrile, *n*-hexane, water, or tissue residue was calculated. The blood was partitioned directly with chloroform and aliquots of each layer were assayed for total radioactivity. The bone was not subjected to this fractionation procedure. Known amounts of radioactive Ruelene were added to the tissues from three untreated hens and fractionated as previously described to determine the percentage recovery of Ruelene in the acetonitrile fraction. The unextractable P^{32} materials from the liver residue were further separated into acid-soluble phosphorus compounds, phospholipids, ribose nucleic acid, and desoxyribose nucleic acid according to methods described by Schneider (1945).

Eggs were collected daily and analyzed separately for total P^{32}-containing materials in the yolk, white, and shell (Dorough *et al.* 1961a). The egg yolk was analyzed for acetonitrile-soluble radioactive materials using the fractionation procedure described for tissues. Analyses of eggs were made in duplicate, and to increase the sensitivity of the radioassay method, from three to four yolks were combined for determining the p.p.m. acetonitrile-soluble materials.

The droppings from each hen were collected at 24-hour intervals after treatment, weighed, mixed thoroughly, and two 500-mg. samples were radioassayed. The p.p.m. Ruelene equivalents and the cumulative percentage of the ingested Ruelene that was eliminated in the excreta were calculated from these analyses. After the total P^{32}-materials in the feces were determined, the fecal samples collected during a given 24-hour period were mixed and three 125-g. subsamples were placed in flasks containing 600 ml. of acetone and allowed to stand about 12 hours. The acetone was decanted and 600 ml. of benzene was added to the solid materials and allowed to stand another 12 hours. The benzene and acetone extracts were combined and processed as described for extracts of tissues. The percentage of the P^{32}-materials occurring in the water, *n*-hexane, acetonitrile, and feces residue was calculated from duplicate analyses of each of the three subsamples of feces.

The radioactive materials in the acetonitrile fraction and in the *n*-hexane fraction of the feces were co-chromatographed on Celite with 50 mg. of nonradioactive Ruelene. The chromatogram was developed, using acetonitrile saturated with *n*-heptane as the immobile phase and *n*-heptane saturated with acetonitrile as the mobile phase (Arthur & Casida 1959). The identical chromatographic behavior of the total phosphorus determined colorimetrically and the total phosphorus determined radiometrically was used to calculate the percentage residual Ruelene and the percentage of other radiophosphorus metabolites.

The radioactive water-soluble metabolites of the feces were subjected to ascending paper chromatography, using untreated paper with acetonitrile and water (85:15) as the mobile solvent (Kaplanis *et al.* 1959). The solvent front was allowed to travel about 30 cm. The radioactive materials were located by counting 1-cm. strips of the chromatograms. All paper chromatograms were run in triplicate on the water-solubles from the feces collected for the first 8 days after initiating the experiment. Aliquots of the water-soluble metabolites were also chromatographed on an anion exchange (Dowex 1×8) column using various hydrochloric acid elution gradients; in certain gradients acetone and methanol were used as co-solvents (Plapp & Casida 1958).

RESULTS.—*Tissue Residues.*—The recovery of 1 to 3 p.p.m. Ruelene added to tissues and eggs from untreated hens were as follows: blood, 97%; brain, 97%; breast, 88%; drumstick, 88%; fat, 89%; gizzard, 70%; kidney, 96%; liver, 90%; skin, 85%; egg yolk, 97%; and egg white, 96%. The acetone and benzene extraction of tissues followed by the *n*-hexane and acetonitrile cleanup procedure (Dorough *et al.* 1961a) was sufficient for removing Ruelene from tissues as evidenced by the above recoveries in the acetonitrile fraction.

The p.p.m. Ruelene equivalents and the p.p.m. acetonitrile-soluble radioactive materials in several tissues of hens are given in table 1. During the 7 days the hens were on Ruelene fortified feed, the highest concentration of total P^{32} residues was found in the liver, kidney, bone, and blood. These residues in the liver, kidney, and blood declined sharply when the hens were returned to normal feed. The occurrence of P^{32} materials in the leg bone was noted after the hens were on radioactive feed for only 24 hours and persisted after the hens were on normal feed for 14 days. There were no detectable P^{32} materials in the fat at any time during or after feeding radioactive feed.

The blood, brain, breast, drumstick, fat, and skin were free of acetonitrile-soluble radioactive materials which indicated that if Ruelene were present in these tissues, the amounts were below the 0.04 p.p.m. sensitivity of the radioassay method. The liver, kidney, and gizzard contained detectable acetonitrile-soluble residues while the hens were on radioactive feed for 7 days, but these residues were dissipated within 3 days after the hens were returned to normal feed. Most of the P^{32} materials remained in the tissue residue following acetone and benzene extraction of tissue homogenates. The unextractable

Table 1.—P.P.M. Ruelene equivalents in tissues of laying hens receiving the insecticide in diet at 100 p.p.m.

Tissue	P.p.m. Ruelene Equivalents at Days Indicated							
	On Ruelene Fortified Feed			After Return to Normal Feed				
	1	3	7	1	3	7	10	14
Blood	0.09	0.41	1.05	0.94	0.75	0.34	0.05	<.04
Bone	.10	.86	1.92	1.42	1.71	1.53	.66	.85
Brain	<.04	.12	.31	.35	.23	.28	.15	.16
Breast	<.04	.20	.56	.59	.60	.53	.23	.21
Drumstick	<.04	.30	.58	.56	.38	.46	.20	.08
Fat	<.04	<.04	<.04	<.04	<.04	<.04	<.04	<.04
Gizzard	.98 (.15)[a]	.52 (.23)	.79 (.20)	.77 (.10)	.43	.17	.06	<.04
Kidney	1.11	2.42 (.16)	3.59 (.16)	2.55 (.15)	1.64	.80	.31	.23
Liver	1.63 (.08)	3.27 (.08)	4.20 (.06)	2.61	1.42	1.10	.44	.21
Skin	.05	.16	.30	.24	.17	.13	<.04	<.04

[a] Numbers in parenthesis indicate the p.p.m. acetonitrile-soluble residues; all other tissues contained below 0.04 p.p.m. acetonitrile-solubles.

P^{32} materials of the liver residue were separated into acid-soluble compounds, phospholipids, RNA and DNA (table 2), and most of the radioactivity was in the acid-soluble fraction.

Residues in Eggs.—Considerably more total P^{32} materials accumulated in the yolk than in the shell of eggs, and none was noted in the white (table 3). Based on total Ruelene equivalents, the peak concentration of radioactivity in egg yolks appeared at 10 to 12 days after initiating the experiment or 3 to 5 days after the hens were returned to normal feed. The acetonitrile-soluble radioactive materials in the yolks followed the same pattern as the total P^{32} residues, except that the quantities present were much smaller. The occurrence of the acetonitrile-soluble residues in the egg yolks (table 3) did not parallel the decline of acetonitrile-soluble residues in the tissues (table 1). The nature of the egg yolk P^{32} residues were not studied further, since the quantities present were too small to characterize by column or paper chromatography.

Table 2.—Results of fractionating the unextractable radioactive materials from liver residue.

Days	Per Cent Present As—			
	Acid Soluble Compounds	Phospholipids	RNA	DNA
On Ruelene feed				
1	98.4	0.0	1.6	0.0
3	80.0	18.8	1.2	0
7	81.3	7.1	11.6	0
After return to normal feed				
1	88.7	6.6	4.7	0

Table 3.—P.P.M. Ruelene equivalents in eggs from hens receiving the insecticide in diet at 100 p.p.m.

Days	P.p.m. Ruelene Equivalents[a]		P.p.m. Acetonitrile-Solubles in Egg Yolk[b]
	Shell	Yolk	
On Ruelene feed			
1	<0.04	<0.04	—
3	<.04	<.04	—
5	<.04	1.13	<0.04
7	.30	2.30	.10
After return to normal feed			
1	0.33	3.75	0.06
3	.20	6.35	.14
5	.13	5.55	.09
7	.12	4.55	.06
10	<.04	1.70	<.04
14	<.04	.94	—

[a] Duplicate analyses on 3 to 6 eggs at indicated days.
[b] Three to four yolks combined for analysis.

Feces.—The percentage of the Ruelene consumed in the feed that was accounted for in the feces as Ruelene equivalents was 17.5% by the end of the first day and 27% at the end of the 7-day feeding period (fig. 1). The average p.p.m. Ruelene equivalents in the feces reached a peak at 4 days after the hens were on radioactive feed and remained at about the same level for the remainder of the 7-day feeding period (fig. 1). As soon as the hens were placed on normal feed, the amount of P^{32} materials in the feces declined rapidly. Almost 30% of the P^{32} consumed was accounted for in the feces by 21 days after initiating the experiment. The amount of feed consumed per day per hen ranged from 45 to 98 gm., which corresponds to a daily intake of 2.91 to 6.22 mg. of Ruelene per kg. of body weight.

Fractionation of the fecal metabolites into acetonitrile, *n*-hexane, and water fractions, and the unextractable materials of the fecal residue yielded the results given in table 4. The amount of P^{32} materials remaining in the feces residue after acetone and benzene extraction ranged from 45% to 68% of the total radioactivity for the 7-day feeding period. When the hens were returned to normal feed, even a larger percentage of the radioactivity remained in the feces residue.

The 1.5% to 5.8% of the radioactivity eliminated in the feces that was present in the acetonitrile fraction was co-chromatographed on Celite with nonradioactive Ruelene (table 5). Only 14% to 38% of these acetonitrile-soluble materials behaved as Ruelene; the remainder was

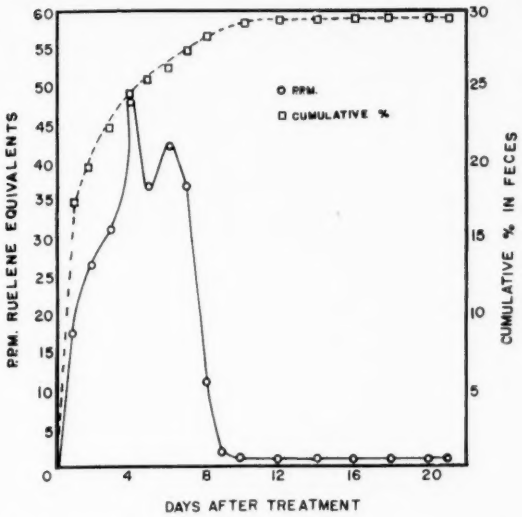

FIG. 1.—P.P.M. Ruelene equivalents and cumulative percentage of the administered dose eliminated in feces of hens receiving the insecticide in the feed at 100 p.p.m.

eluted from Celite in the methanol fraction. Less than 1% of the radioactive materials in the *n*-hexane fraction was Ruelene. The nature of the metabolites represented by the methanol fraction from Celite represents Ruelene degradation products that were extractable from water into *n*-hexane, but were too polar to be eluted from Celite with the regular mobile solvent of *n*-hexane saturated with acetonitrile.

The rapid degradation of Ruelene was quite evident from the percentage of the radioactivity remaining in the water fraction and feces residue following acetone and benzene extraction (table 4). When the water-soluble metabolites were chromatographed on paper strips, six and possibly nine radiophosphorus metabolites were isolated (table 6). The predominate metabolites had an Rf of 0.36 and 0.86. None of the products listed in table 6 was Ruelene, since this material was removed from the feces in the extraction process, and none of the materials had an Rf of known Ruelene (0.94). Four suspected metabolites of Ruelene were provided by The Dow Chemical Company: (I) 4-*tert*-butyl-2-chloro-phenyl phosphoric acid, (II) *O*-methyl N-methyl amidophosphoric acid, (III) 4-*tert*-butyl-2-chlorophenyl methylamidophosphoric acid, and (IV) 4-*tert*-butyl-2-chlorophenyl methylphosphoric acid. The radioactive material with an Rf of 0.55 was tentatively identified as 4-*tert*-butyl-2-chlorophenyl methyl phosphoric acid. The unknown with an Rf of 0.06 was believed to be *O*-methyl phosphoric acid (known material provided by the Victor Chemical Company). Some of the unknown spots (table 6) possibly contained two radioactive materials, and the possibility that other suspected degradation products were present cannot be excluded.

DISCUSSION.—The biological instability of Ruelene is quite apparent based on the number of metabolites recovered from poultry feces (tables 5 and 6). Although only two of the degradation products were tentatively identified, it appears that Ruelene is degraded at the P-N and P-O-C groupings. This view has been supported by subsequent research with sheep (Timmerman *et al.* 1960)

Table 4.—Results of extracting Ruelene and metabolites from feces of hens receiving the insecticide in diet at 100 p.p.m.

	Per Cent of Radioactivity Present in—			
Days	Acetonitrile	*n*-Hexane	Water	Feces Residue
On Ruelene feed				
1	1.8	0.3	49.9	48.0
2	2.3	.2	51.9	45.6
3	1.6	.1	43.8	54.5
4	2.1	.4	38.1	59.4
5	3.7	.3	27.3	68.7
6	3.0	.4	38.7	57.9
7	2.9	.3	53.7	43.1
After return to normal feed				
1	5.8	.6	46.0	47.6
2	2.6	.3	21.6	75.5
3	1.5	.8	9.4	88.3

Table 5.—Celite partition chromatography of acetonitrile and *n*-hexane-soluble materials recovered from feces of hens receiving Ruelene in diet at 100 p.p.m.

	Per Cent of Radioactivity Present as—			
	Acetonitrile-Solubles		*n*-Hexane-Solubles	
Days	Ruelene	MeOH Fraction	Ruelene	MeOH Fraction
On Ruelene feed				
1	14.6	85.4	<1.0	>99
3	33.9	66.1	<1.0	>99
5	31.7	68.3	<1.0	>99
7	38.1	61.9	<1.0	>99
After return to normal feed				
1	37.9	62.1	<1.0	>99

Table 6.—Separation of Ruelene water-soluble metabolites by paper chromatography.

	Rf and Per Cent of Radioactivity Present as[a]—					
Days	0.06[b]	0.23	0.36	0.55[b]	0.75	0.86[b]
On Ruelene feed						
1	<1.0	1.2	29.9	5.3	23.4	40.2
2	<1.0	1.3	29.3	9.1	24.3	36.0
3	2.3	1.8	24.8	13.9	9.8	47.4
4	<1.0	<1.0	53.1	11.9	7.6	27.5
5	1.0	1.0	40.6	20.6	5.3	31.4
6	<1.0	<1.0	56.9	16.0	5.0	22.1
7	<1.0	<1.0	64.3	12.7	12.6	10.5

[a] Compound with Rf of 0.06 tentatively identified as *O*-methyl phosphoric acid and 0.55 as 4-tert-butyl-2-chlorophenyl methyl phosphoric acid.
[b] Possibly two materials.

where *O*-methyl N-methyl amidophosphoric acid and 4-*tert*-butyl-2-chlorophenyl phosphoric acid were identified in addition to the *O*-methyl phosphoric acid and 4-*tert*-butyl-2-chlorophenyl *O*-methyl phosphoric acid isolated from poultry feces. The acetone and benzene extraction of the feces left a considerable portion of the total radioactivity in the fecal material and this could account for the absence of some metabolites that were identified from sheep excreta. Then too, the methanol fractions of Celite columns contained Ruelene degradation products that were not identified. The degradation scheme of Ruelene is complex and some of the metabolites are probably transitory, since there was a wide variation in the percentage of metabolites formed based on anion exchange columns and paper chromatograms of the water-soluble products of the feces. No doubt some of the Ruelene metabolites were not stable in the hydrochloric acid elution gradients used on the anion exchange columns.

The rapid incorporation of Ruelene metabolites in the bone tissue suggests a complete breakdown to phosphoric acid. The complete hydrolysis of this organophosphate to phosphoric acid and the tremendous metabolic pool requiring this essential acid probably accounts for the low percentage of the consumed dose that was eliminated in the feces (29%) as opposed to 75% for Co-Ral (Dorough *et al.* 1961b).

The recoveries of known amounts of Ruelene added to poultry tissues and eggs were excellent, but all radioactive materials in the acetonitrile fraction of tissues from hens receiving Ruelene in the diet could not be classified as Ruelene. Some of the less polar degradation products of Ruelene could have partitioned from water into *n*-hexane and then into the acetonitrile fraction. This appeared to be the case with the radioactive materials of the egg yolks, since acetonitrile-soluble residues occurred in yolks after the hens were returned to normal feed and after the acetonitrile-soluble P^{32} materials had disappeared from the tissues. The occurrence of apparent Ruelene residues in egg yolks was similar to the residue pattern of Co-Ral (Dorough *et al.* 1961a, 1961b) and malathion (March *et al.* 1956).

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The Effects of DDT on Oxidative Metabolism in Susceptible and DDT-Resistant *Aedes aegypti*[1,2]

DON W. MICKS and M. R. V. MURTHY, *Laboratory of Medical Entomology, Department of Preventive Medicine and Public Health, University of Texas Medical Branch, Galveston*

ABSTRACT

Susceptible (S) *Aedes aegypti* (L.) larvae exhibited a higher respiratory rate than those of a DDT-resistant (R) strain. Whereas various concentrations of DDT increased the rate of oxygen consumption of R larvae, low concentrations of the insecticide increased oxygen uptake of the S strain and high concentrations decreased it. When the temperature was increased from 28° C. to 37.1° C., the oxygen consumption of the R larvae was higher. As compared with intact larvae, homogenates exhibited a prolonged lag period in their oxygen uptake, after which oxygen was consumed at a greatly enhanced rate, which was higher in the S larvae.

The addition of the soluble fraction of the cell increased the oxygen uptake of mitochondria even when present in a concentration of only one-fifth that of the total homogenate.

Although DDT inhibited oxidative phosphorylation in both strains, the degree of inhibition was greater in the S strain. This inhibition was due largely to the reduction in the esterification of inorganic phosphate, the oxygen uptake being only slightly affected.

Comparative studies of susceptible and insecticide-resistant strains of insects have frequently failed to show consistent differences in oxygen consumption or in cellular respiration associated with specific enzyme activity which could be related to resistance, although the addition or application of an insecticide may elicit specific differences. For example, Fullmer & Hoskins (1951) found that when DDT was applied topically to susceptible and resistant house flies which normally respire at equal rates, the peak oxygen consumption of the susceptible strain was two to three times higher and occurred much sooner than that of the resistant strain. More recently, DDT has been shown to exert specific effects upon subcellular respiration. Sacklin *et al.* (1955) reported that DDT inhibited the oxidation of citric acid cycle intermediates and oxidative phosphorylation when these processes were catalyzed by subcellular particles from adult house flies (*Musca domestica* L.). Likewise, it has been determined that DDT markedly reduced the oxidation of α-ketoglutarate and uncoupled phosphorylation in a sarcosome preparation from adult *Aedes aegypti* (Gonda *et al.* 1957). The results of these and other investigations show that DDT directly affects oxidative metabolism and that the effects *in vivo* may not be interpreted as the indirect consequences of neuromuscular disturbances (Winteringham & Lewis 1959).

Due to the paucity of such information concerning mosquitoes, it was of interest to study the effects of DDT on the oxygen consumption of susceptible and DDT-resistant *Aedes aegypti* larvae and homogenates as well as the effects of DDT on oxidative phosphorylation in mitochondria.

MATERIALS AND METHODS.—Fourth-stage larvae of *Aedes aegypti* (L.) were used throughout this work. A susceptible strain (UTMB) with a DDT LC_{50} of 0.5 p.p.m., is referred to hereafter as the S strain and a DDT-resistant strain (Trinidad), with an LC_{50} of 2.5 p.p.m. DDT, is herein designated the R strain.

Oxygen uptake by larvae, homogenates and subcellular particles was determined with conventional Warburg manometric techniques. Air was the gas phase in all cases. Duplicate flasks were used in all measurements and whenever a large variation was noticed the experiment was repeated. Larvae of the same size were selected, and both wet and dry weights of a given number of these were determined to assure that all batches in a single experiment were as near equal in weight as possible. The *in vivo* experiments were conducted with 25 larvae per flask, suspended in 3 ml. of distilled water. For the *in vitro* studies, homogenates representing the same number of larvae per flask were distributed in 3 ml. of 0.01 Tris (hydroxymethyl) aminomethane buffer, pH 7.2. No external substrate was present in either case. Mitochondria used for oxygen uptake measurements were suspended in 3 ml. of 0.01 M Tris buffer (pH 7.2) containing 10 mg. of sodium succinate at a temperature of 24.5° C. Whenever DDT was added, an appropriate quantity of an acetone solution of this insecticide (2.2×10^{-3} M) was placed in the side arm of the Warburg flask and was then dispersed in the aqueous phase by adding 0.5 ml. of water. The controls were treated with an equivalent volume of acetone. After 30 minutes, this suspension was tilted into the main compartment to give a final concentration of 1.1×10^{-4} M. The total volume was 3 ml. in all cases.

Table 1.—Effect of DDT on oxidative phosphorylation in mitochondria from DDT-susceptible (S) and DDT-resistant (R) larvae of *A. aegypti*.

Enzyme Source	O_2 Uptake (μG. atoms/30 min.)	Inorganic-P Esterified (μG. atoms/30 min.)	Ratio P:O
S Mitochondria only	7.5	20.8	2.77
S Mitochondria + DDT	7.2	8.3	1.15
R Mitochondria only	7.6	18.5	2.43
R Mitochondria + DDT	5.8	8.8	1.52

For the preparation of subcellular fractions, the larvae were washed several times with double distilled water and then homogenized in a glass tissue grinder in the cold room at the temperature of ice with 5 volumes of an extraction medium containing 0.33 M sucrose, 0.6% bovine serum albumin and 10^{-4} M ethylene diamine tetra-acetic acid. The mitochondria were sedimented by

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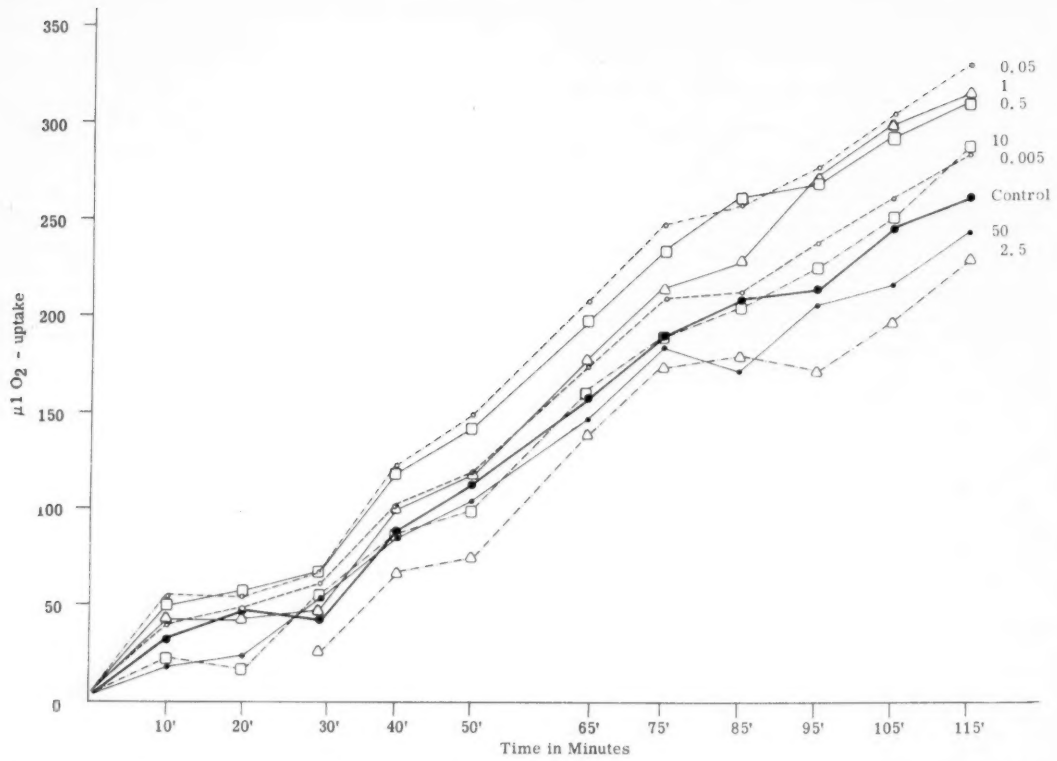

FIG. 1.—Effect of DDT (p. p. m.) on the oxygen uptake of DDT-susceptible *A. aegypti* larvae *in vivo*. Temperature —24.5° C.

centrifuging at 8000 *g* for 7 minutes after removing the unbroken cells and cell fragments at 600 *g*. The mitochondrial pellets were washed three times before suspending them in buffer. The soluble fraction was obtained by centrifuging the homogenate as prepared above at 105,000 g. for 90 minutes. The supernatant was diluted with 0.01 M Tris buffer to the concentration desired. Studies of oxidative phosphorylation in mitochondria utilized a mixture of 0.22 M sucrose, 0.1% albumin, 10^{-4} M EDTA, 100 μmoles of Tris buffer (pH 7.4), 40 μmoles of phosphate buffer (pH 7.4), 8 μmoles of ATP, 8 μmoles of $MgCl_2$, 0.33 μmoles of DPN, 30 μmoles of α-ketoglutarate and mitochondria equivalent to 0.5 gm. of wet weight of larvae. Glucose (100 μmoles) and 100 K.M. units of hexokinase (Kunitz & McDonald 1946) were added from the sidearm after incubation for 7 minutes at 28° C. The total volume upon the addition of all components was 3 ml. The center well contained 0.2 ml of 20% KOH. The final concentration of DDT was 1.1×10^{-4} M. The incubation temperature was 28° C. Oxidative phosphorylation and the simultaneous uptake of oxygen were measured according to the procedure described by Hunter (1955). Inorganic phosphate was determined by the method of Lowry & Lopez (1946).

RESULTS AND DISCUSSION.—Figure 1 shows that DDT, up to a level of 1 p.p.m., increased the rate of oxygen uptake of the S larvae and at higher concentrations decreased it. The only exception to this was the rate of uptake at the 10 p.p.m. concentration which closely followed that of the untreated larvae. This latter response could not be accounted for. In the R strain (fig. 2), all concentrations of DDT from 0.5 p.p.m. to 50 p.p.m. increased the rate of oxygen consumption. It was also noted that at each level of DDT added, the uptake was higher in the S strain than in the R strain. However, even in the absence of DDT, the S larvae exhibited a higher respiration rate than the R larvae, under the conditions of this experiment. On the other hand, figure 3 shows that when the temperature was increased from 28° C. to 37.1° C., the oxygen uptake by the R larvae was higher. This experiment was repeated and yielded similar results.

When homogenates of both strains were used, there was a long lag period of approximately 4 hours before oxygen was absorbed to any appreciable extent (fig. 3). At the end of this period, however, oxygen was consumed at a rapid rate, the rate being greater in the S larvae. Thus at the end of 7 hours at 37.1° C., although the total oxygen consumed by the S larvae *in vivo* was about 30% lower than that of the R larvae, the total oxygen uptake by an equivalent quantity of the homogenates was approximately 45% higher in the S strain.

With the data presently available, it is difficult to explain the 4-hour lag period and the subsequent rapid consumption of oxygen. Plate tests with homogenates after incubation for 0 hr., 4 hrs. and 7 hrs. in Warburg flasks at 37.1° C. under the same conditions showed that

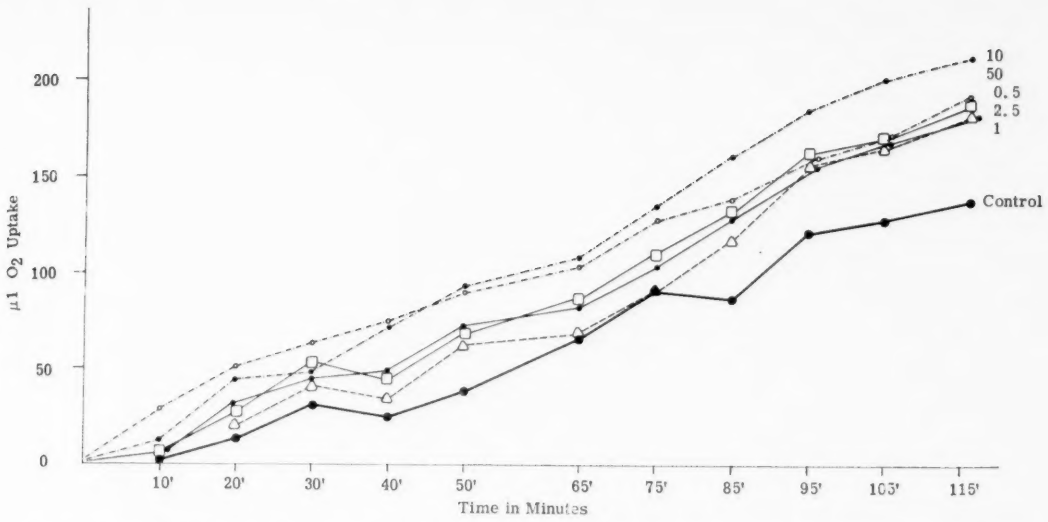

FIG. 2.—Effect of DDT (p. p. m.) on the oxygen uptake of DDT-resistant *A. aegypti* larvae *in vivo*. Temperature —24.5° C.

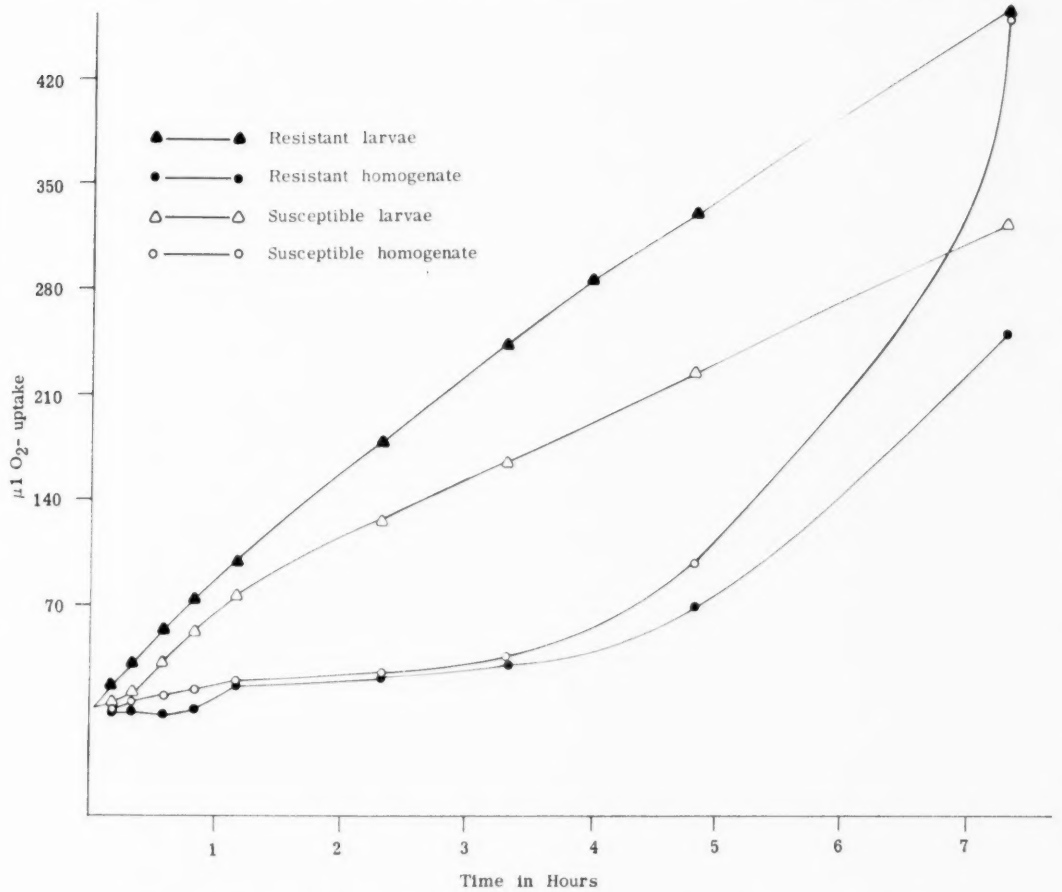

FIG. 3.—Effect of homogenization on the oxygen uptake of susceptible and DDT-resistant strains of *A. aegypti* larvae. Temperature —37.1° C.

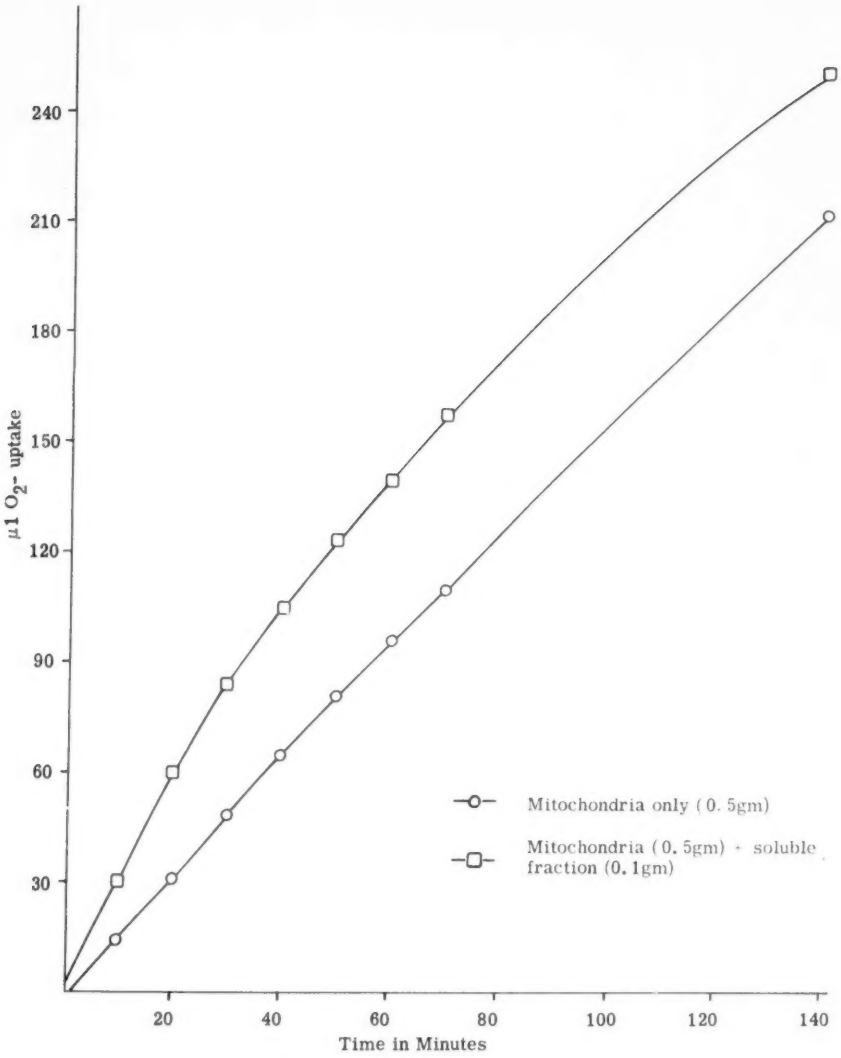

FIG. 4.—Effect of the soluble fraction on the oxygen uptake of mitochondria from the DDT-resistant *A. aegypti* larvae. Temperature —24.5° C.

there was no demonstrable bacterial growth during the experimental period. It is to be noted that the endogenous substrates of the larval homogenates become distributed throughout the volume of the reaction mixture, diluting their concentration considerably, whereas in the intact larvae, this concentration remains the same irrespective of the volume of the aqueous phase in which the larvae are suspended. The release of nucleotidases and ATPase into the reaction mixture by homogenization may also play a part in this phenomenon. For example, mitochondria are known to possess a powerful ATPase activity which becomes active upon incubation. This in turn produces adenosine diphosphate (ADP), which being the primary phosphate acceptor, controls mitochondrial oxidations (Schneider 1959). Oxidation of the substrate would be expected to proceed at an accelerated rate as soon as the required level of ADP is reached. Furthermore, it has been observed that homogenates of larvae incubated for long periods release large quantities of free amino acids and possibly other compounds (Micks & Murthy 1961) which might serve as additional substrate for respiration as suggested by Winteringham (1958).

Figure 4 shows that the addition of as little as 0.1 gm. larval equivalent of the soluble fraction of the cell to 0.5 gm. equivalent of mitochondria increases the oxygen uptake appreciably. Similar effects of the supernatant fraction on the oxygen consumption of mitochondria have been reported by other workers (Hogeboom & Schneider

1950, Siekevitz 1952). This phenomenon is attributed to the fact that the cell metabolites required for the complete oxidation of the citric acid cycle substrates are distributed between different subcellular fractions (Schneider 1959). Therefore, it seems reasonable to assume that the maximum oxygen uptake is the result of the integrated action of mitochondria with other subcellular components.

Using α-ketoglutaric acid as the substrate, it was found that the S mitochondria gave a slightly higher P:O ratio than the mitochondria of the R strain (table 1). Whereas there was an inhibition of oxidative phosphorylation equal to 58% in the S strain in the presence of DDT, there was only 37% inhibition in the R strain. The uptake of oxygen during the 30-minute experimental period was affected only slightly (a reduction of 4% in the S strain and 23% in the R strain), but the esterification of inorganic phosphate was drastically reduced (60% in the S strain and 52% in the R strain). This is in conformity with the findings of Gonda *et al.* (1957), who reported that in their strain of *A. aegypti* adults, phosphorylation was more affected by DDT than was respiration.

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Phoresy in *Xenufens* (Hymenoptera: Trichogrammatidae), A Parasite of *Caligo eurilochus* (Lepidoptera: Nymphalidae)[1]

FEDERICO MALO,[2] *Department of Research, Compania Bananera del Ecuador, Guayaquil*[3]

ABSTRACT

Xenufens sp. near *ruskini* Girault parasitizes the eggs of the butterfly, *Caligo eurilochus* (Cramer), a pest of banana in Ecuador. Adults of this wasp have been found riding on the hind wings of both sexes of the host. During oviposition the butterfly holds its hind wings erect over the body in contact with its abdomen and ovipositor; this action brings the parasite close to the host's eggs, and presumably it is at this time that the eggs are parasitized. Details of this association together with data on the control effected by the parasite are given.

Phoresy has been reported for adults of certain scelionid and trichogrammatid egg parasites (Sweetman 1958). The trichogrammatid *Oligosita xiphidii* Ferrière has been found clinging to the hind wings of the tettigoniid *Xiphidion longipenne* de Haan, in Java (Ferrière 1926). As far as the writer is aware, the present note is the first report of phoresy by *Xenufens* on adults of the butterfly *Caligo*.

Specimens of *Xenufens* sp. near *ruskini* Girault were reared from eggs of *Caligo eurilochus* (Cramer) (possibly race *morpheus* Stichel), and adults of the same wasp were also collected from the hind wings of this species of *Caligo*. These wasps were found for the first time as adults in Tenguel, Ecuador, by Dr. R. C. Bullock during November 1956.

Adults of *Xenufens* are carried on the interior anal margin (mainly in the anal angle) of the hind wings of both females (11 of 13 examined) and males (79 of 100) of *C. eurilochus*. From 1 to 120 *Xenufens* have been collected on a single male *Caligo*, but the average number of wasps found per male was 14.6 ± 2.4 ($N = 79$). Though adult females of *Caligo* are less prevalent than males in the field (ratio 1:8), the former may carry from 1 to 250 adult wasps, with an average of 78.5 ± 25.4 per individual ($N = 11$).

During oviposition, the wings of *Caligo* are folded in an erect position which allows the anal margins of the hind wings to come in contact with the abdomen and ovipositor. Oviposition usually requires several minutes, and

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[3] A subsidiary of United Fruit Company.

Table 1.—Parasitization of *Caligo* eggs by *Xenufens* in Tenguel, Ecuador, from November 1958 to October 1959.[a]

	1958				1959																	
	Nov.		Dec.		Jan.		Feb.		March		April		May		June		July		Aug.		Sept.		Oct.	
FARM	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
S. Fran.	73	88	21	100	18	100	15	47	X		5	20	37	46	46	100	108	94	125	78	266	99	101	79
Papayos	X		X		8	88	10	100	X		X		11	100	X		13	92	31	97	13	100	4	100
Pichin.	30	77	41	30	28	64	27	52	13	62	X		10	100	9	89	17	41	66	100	20	100	63	65
Balarez.	4	100	X		X		9	100	X		X		16	94	13	100	X		25	96	X		X	
Esperza.	18	100	X		4	100	X	X	X		X		X		6	83	6	100	99	52	66	98	13	100
Sangay	50	100	83	94	36	22	14	100	7	100	18	50	36	69	43	100	107	87	65	97	65	98	16	100
Chimbo.	20	100	28	78	18	100	12	75	8	100	28	50	44	48	12	100	68	72	57	88	74	39	25	84
S. Rafa.	—	—	—	—	—	—	—	—	—	—	—	—	18	100	15	100	7	100	36	100	6	50	20	100
Illinza.	—	—	—	—	—	—	—	—	—	—	—	—	72	90	42	95	47	100	63	79	61	16	23	100
Mean percentage parasitized of all eggs examined		92		77		65		72		82		47		75		98		86		82		82		82

[a] No. = Number of *Caligo* eggs examined. % = Percentage of eggs parasitized. X = No eggs found during the survey. — = No survey.

it is undoubtedly at this time that *Xenufens* has access to and parasitizes the butterfly eggs. How, or when, *Xenufens* attaches itself to the *Caligo* adult is unknown. The wasps may be attracted to the adult butterfly and attach themselves to females and later transfer to males during copulation. It is conceivable that once a male butterfly becomes a carrier of *Xenufens*, it may assist in dispersing the wasp by mating with several females, if, during the act of copulation, the trichogrammatid transfers from the male to the female.

The eggs of *Caligo eurilochus* usually are laid on the under surface of banana leaves and sometimes on the upper part of the pseudostem. The egg is subspherical, strongly ribbed, pearly white, and about 1.7 to 2.0 mm. in diameter and height. The pole attached to the leaf is flattened. Unparasitized, normally developing eggs remain creamy in color; they hatch in about 10 days after laying. If parasitized, the creamy-white egg changes through light gray to deep gray-black in 4 to 6 days; the adult of *Xenufens* emerges about 13 days after parasitization of eggs (based on egg masses not over 24 hours old when collected). Most of the *Caligo* egg masses found consisted of about 7 eggs, but there may be as many as 20; the eggs may also be deposited singly. Usually, among egg masses consisting of a large number of eggs, only some of the eggs are parasitized. Up to 40 *Xenufens* adults have been reared from one *Caligo* egg with an average of 34.3 ± 1.5 ($N = 7$).

When parasitized *Caligo* eggs were dissected, a maximum of 43 *Xenufens* larvae were found, with an average per egg of 33.9 ± 2.1 ($N = 9$). The wasp larva is oval in shape and cream-colored. As many as 52 *Xenufens* pupae were found in one *Caligo* egg with an average of 28.7 ± 5.5 ($N = 6$); the pupae have cream-colored bodies and deep red eyes.

Usually the adults of *Xenufens* emerge through a small round opening made at the apex of the egg opposite the pole attached to the leaf. However, occasionally the opening is made along the equator of the egg shell. At times individuals of *Xenufens* may develop and mature inside the *Caligo* egg but for unknown reasons they remain as mummified adults inside the egg shell. As many as 29 mummies (average of 20.7 ± 2.8, $N = 7$) have been found inside *Caligo* eggs.

A mite, *Tyrophagus* sp. (Acaridae), has been found associated with *Xenufens* inside the *Caligo* eggs. *Xenufens* larvae dissected from *Caligo* eggs have had 2 to 4 of these mites in them. The mites were also collected in some of the mummified adults of *Xenufens* that had not emerged from the butterfly eggs. Some species of *Tyrophagus* are pests of stored foods and certain crops. In general members of the Acaridae live on a variety of organic substances, including dead and decaying insects. Except for one genus, *Thyreophagus*, in this family, none have been found to be either predaceous or parasitic (Baker & Wharton 1952). *Xenufens* did not emerge from 50% of the 100 parasitized *Caligo* eggs observed.

An indication of the extent of control of *Caligo* by *Xenufens* in the banana plantations in Tenguel is shown in table 1. During the 12-month period an average of 82% of the eggs examined were parasitized.

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Recent Developments in the Insecticidal Control of the Apple Maggot[1]

R. W. Dean, *New York State Agricultural Experiment Station, Poughkeepsie*

Abstract

Chemicals exhibiting a high degree of toxicity to adults of the apple maggot, *Rhagoletis pomonella* (Walsh), in laboratory tests included Dow M361, Dow ET-14, Dow ET-15, Diazinon®, Pirazinon®, Dipterex®, endrin, isodrin, ethion, Thiodan®, Bayer 25141, phosphamidon, Dibrom®, Phosdrin®, Guthion® and dimethoate. Of these, Dow ET-14, Dow ET-15, Diazinon, Pirazinon, Dipterex, endrin and isodrin brought about the most rapid kill. Other materials tested in the laboratory that showed good to moderate toxicity to the flies were Chlorthion®, DDVP, Sevin®, Trithion® and Di-Syston®. Phostex® and Ryania were the least effective insecticides used in the laboratory tests.

In orchard trials, insecticides were applied as foliage sprays timed according to fly emergence. Isolated orchards, receiving a single schedule of treatments throughout the entire block, were used, as well as multiple-plat experiments that included unsprayed check plats. Migrant flies did not present a problem in plats buffered by a single row of sprayed trees. Spray schedules employing a combination of lead arsenate and Perthane®, Diazinon alone and combined with DDT, DDT plus lead arsenate, Guthion alone, and Guthion plus Sevin, or Guthion plus lead arsenate were effective when used in practicable amounts. Sevin and dimethoate prevented most larval development within the fruit but permitted puncturing by ovipositing flies and, therefore, are not considered acceptable for commercial use at the rates tested. Strobane® and Bayer 22408, while reducing injury, did not provide sufficient protection to warrant further trial. A combination, at reduced levels, of Trithion and DDT, also gave inadequate control, but a schedule including DDT, alone and combined with lead arsenate, with one application of Trithion alone was effective. As used in these tests, phosphamidon, ethion, malathion, Diazinon in a two-application schedule, and Diazinon combined with malathion failed to control apple maggot. Sevin combined with lead arsenate failed in one test but, when Guthion was substituted in one application, gave satisfactory control in another. Most failures, under orchard conditions, appear to result from inadequate residual lethal action.

The chemical names for the insecticides mentioned above for which no approved common names are available are as follows:

Bayer 22408—*O,O*-diethyl *O*-naphthalimido phosphorothioate
Bayer 25141—*O,O*-diethyl *O*-*p*-(methylsulfinyl) phenyl phosphorothioate
Chlorthion—*O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate
Diazinon—*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate
Dibrom—1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Dipterex—dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate
Di-Syston—*O,O*-diethyl *S*-2-(ethylthio) ethyl phosphorodithioate
Dow ET-14—*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate
Dow ET-15—*O*-methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate
Dow M361—*O*-methyl *O*-2,4,5-trichlorophenyl *N*-ethyl phosphoramidothioate
Guthion—*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate
Perthane—1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane
Phosdrin—1-methoxycarbonyl-1-propen-2-yldimethyl phosphate (and related compounds)
Phostex—a mixture of bis (dialkyloxyphosphinothioyl) disulfides
Pirazinon—*O,O*-diethyl-*o*-3-(2-propyl-6-methyl-4-pyrimidinyl) phosphorothioate
Sevin—1-naphthyl *N*-methylcarbamate
Strobane—a mixture of chlorinated terpenes with 66% chlorine
Thiodan—6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Trithion—*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate

Three previous reports have been made by the writer (Dean 1947, 1951, 1954) on the results of laboratory and field tests of insecticides against the adult stage of the apple maggot, *Rhagoletis pomonella* (Walsh). The present paper continues the series to cover work done from 1954 through 1960. The experimental methods used are, for the most part, the same as those previously described. In the laboratory, two kinds of tests were employed, which are termed, respectively, *discontinuous exposure* and *controlled exposure* tests. In the first, an insecticidal film is applied to a glass plate forming the top of the cage in which the insects are confined. Flies are in contact with the film only when walking on that one side of the cage. Mortality records thus obtained express simple dosage-mortality and time-mortality relationships. For the controlled exposure tests, the insecticide is applied evenly to both halves of a petri dish and allowed to dry, after which the test insects are confined in it for periods varying from 5 to 60 minutes, and then transferred to clean cages. These tests measure the speed of action of the material at a given dosage level. In both instances, mortality was recorded on a daily basis over a 10-day period.

Field tests were usually made on small, infested blocks of apple trees which were more or less isolated from other host trees. A single material, or combination of materials, was applied to the entire test block without leaving any untreated check trees. The degree of maggot infestation was determined by examination of all fruit from approximately six trees, and compared with the degree of fruit infestation observed the previous year. Treatments were applied with a high-pressure sprayer by means of hand guns operated from the ground. Control operations were timed according to fly emergence for each season, with a definite interval between applications, varying from 10 to 14 days, and continuing as long as significant numbers of flies emerged. The procedure was varied in some tests, application being by means of an air-blast attachment to a high-pressure sprayer in some instances while, in others, a four-nozzle broom was used by an operator riding on top of the spray tank. The type of coverage thus obtained was more like that resulting from commercial orchard

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Table 1.—Results of laboratory tests on apple maggot adults, 1954–1960.

Material	Exposure: Discontinuous — Pounds in 100 Gal.	Mortality (%) Accumulated in (days): 1	5	10	Exposure: Controlled — Pounds per 100 Gals.	Exposure (min.)	Mortality (%) Accumulated in (days): 1	5	10
25% Dow M361	0.125	63	100		0.5	5	2	10	22
	0.25	98	100 (3)[a]			10	12	14	26
Check	0	0	2			15	2	2	25
	0.5	90	100 (2)			30	82	90	98
	1	100				60	13	20	33
Check	0	2	4		0	0	0	0	10
					1	5	29	81	87
						10	66	98	100
						15	100		
Check					0	0	0	8	25
25% Dow ET 14	0.125	65	100 (2)		0.5	5	35	57	69
	0.25	90	100 (2)			10	74	92	92
	0.5	100				15	100		
Check	0	0	2		0	0	0	0	10
					1	5	100		
Check					0	0	0		
25% Dow ET 15	0.125	72	98	100 (8)	0.5	5	25	39	45
	0.25	88	98	100 (8)		10	73	85	90
	0.5	100				15	90	96	98
						30	100		
Check	0	0	2	15	0	0	0	0	10
					1	5	86	100 (3)	
						10	96	100 (2)	
						15	100		
Check					0	0	0	8	25
25% Pirazinon	0.125	82	96	98					
	0.25	98	100 (2)						
	0.5	100			0.5	5	100		
Check	0	0	8		0	0	0		
					1	5	100		
Check					0	0	2		
25% Diazinon	0.125	93	100 (3)		1	5	37	45	45
	0.25	100				10	76	84	84
						15	91	94	94
						30	100		
Check	0	0	8		0	0	2	4	17
					2	5	100		
Check					0	0	0		
50% Dipterex	0.5	100			2	5	100		
50% Chlorthion	0.5	80	98	98	2	5	4	9	16
	1	30	78	88		10	11	24	30
	2	79	97	100 (6)		15	26	38	42
	4	92	100 (3)			30	48	60	60
						60	75	83	83
Check	0	15	33	39	0	0	2	8	18
5% DDVP	0.5	33	61	61	2	5	40	47	47
	1	66	81	83		10	53	67	69
	2	86	98	98		15	55	67	67
	4	98	100 (2)			30	94	100 (2)	
	5	100				60	96	100 (2)	
Check	0	0	17	40		0	0	4	4

(Continued on next page)

[a] Figures in parentheses are actual day on which complete mortality was attained.

Table 1.—*(Continued)*

Material	Exposure: Discontinuous — Pounds in 100 Gal.	Mortality (%) Accumulated in (days): 1	5	10	Exposure: Controlled — Pounds per 100 Gals.	Exposure (min.)	Mortality (%) Accumulated in (days): 1	5	10
10% Endrin	0.5	55	100 (2)		2	5	100		
	1	94	100 (2)						
	2	93	100 (2)						
	4	100							
10% Isodrin	0.5	63	100 (3)						
	1	94	100 (2)		2	5	96	100 (2)	
	2	100				10	100		
Check	0	0	12		0	0	2	12	
50% Sevin	0.25	14	63	67	1	5	17	70	77
	0.5	16	66	74		10	23	50	56
	1	14	92	100 (6)		15	18	65	71
	2	22	94	100 (7)		30	19	40	48
	4	38	100			60	77	90	90
Check	0	2	2	64	0	0	16	100	
25% Ethion	0.5	96	100 (2)						
	4	84	100 (2)						
Check	0	2	2						
25% Thiodan	0.5	24	84	94					
	1	78	100 (3)						
	2	100							
25% Trithion	0.25	65	100 (3)						
	0.5	86	100 (2)						
	4	96	100 (2)						
Check	0	6	14	43					
25% Di-Syston	0.5	68	100 (3)						
	1	94	100 (2)						
	4	100							
25% Phostex	0.5	0	18	84					
	1	0	83	100					
	2	18	100 (4)						
	4	43	100 (3)						
50% Bayer 22408	0.25	0	100						
	0.5	0	100 (4)						
	1	4	100 (3)						
	2	6	100 (3)						
	4	44	100 (3)						
Check	0	0	4	22					
50% Bayer 29493	0.25	96	100 (3)						
	4	96	100 (3)						
50% Bayer 25141	0.25	100							
	4	98	100 (2)						
Check	0	89	100 (4)						
Vapor toxicity:									
50% Bayer 29493	2	96	100 (2)						
50% Bayer 25141	2	64	100 (2)						
Check	0	13	68 (3)						
Phosphamidon 4	0.25 pt.	98	100 (2)						
	1 qt.	86	100 (3)						
Dibrom 8	0.25 pt.	96	100 (2)						
	0.5 pt.	98	100 (2)						

(Continued on next page)

Table 1.—(*Continued*)

Material	Exposure: Discontinuous — Pounds in 100 Gal.	Mortality (%) Accumulated in (days) 1	5	10	Exposure: Controlled — Pounds per 100 Gals.	Exposure (min.)	Mortality (%) Accumulated in (days) 1	5	10
	1 qt.	94	100(2)						
Check	0	30	76(2)						
95% Ryania, Micro.	4	2	96	98					
	6	2	51	69					
	8	2	52	78					
100% Ryania, std.	2	2	96	100(9)					
10% Phosdrin	0.25	65	100(2)						
	0.5	96	100(2)						
	2	100							
Check	0	16	100(3)						
25% Guthion	0.25	75	100(4)		1	5	67	78	82
	1	82	100(2)			10	55	82	88
	2	90	100(4)			15	94	100(2)	
						60	98	100(2)	
50% dimethoate	0.25	87	100(2)		1	5	98	100(2)	
	0.5	90	100(2)			10	92	100(3)	
	1	92	100(2)			15	98	100(3)	
	2	92	100(2)			30	100		
Check	0	2	11		0	0	6	34	64

spraying. Larger test blocks were also used, with treatments applied to one-half of the block only, or different materials were compared on the two halves. Trees counted were selected from those well away from the plat boundaries, and no evidence of fly migration from one area to the other has been apparent. Finally, test orchards have been divided into plats of from 10 to 15 trees, with sprayed buffer rows between the plats, and with untreated check plats left for comparison. There is a limit to the amount of reduction to which the plats can be subjected, but the small-plat technique has proved feasible when testing modern insecticides for apple maggot control, probably because their toxic action is rapid enough to prevent significant amounts of injury by migrating flies. Records from the multiple-plat test blocks were taken by examining randomized samples of 300 apples at each picking or drop-collection except when fewer than that number were available. In such instances, all were counted and examined.

The materials that were tested in the laboratory for toxicity and speed of action, or in the field as foliar sprays, against *R. pomonella* adults, were supplied by various manufacturers in formulations that were considered suitable for application to fruit trees. No attempt was made to compare them on the basis of equal active ingredient content but, rather, a range of dosages was selected around a mean that, from preliminary evidence, appeared to be a practicable amount.

The materials reported on in this series of tests are listed at right.

Bayer 22408—50% *O,O*-diethyl *O*-naphthalimido phosphorothioate wettable powder.

Bayer 25141—50% *O,O*-diethyl *O*-*p*-(methylsulfinyl) phenyl phosphorothioate wettable powder.

Bayer 29493—50% *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate wettable powder.

Chlorthion®—25% *O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate wettable powder.

DDT—50% commercial grade wettable powder.

DDT-Diazinon E.C. #455—Emulsifiable concentrate containing 2 lb. actual DDT and 1 lb. actual Diazinon per gallon.

DDVP—5% wettable powder formulation.

Diazinon®—25% *O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate wettable powder.

Dibrom®—64.5% 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate, (emulsifiable concentrate containing 8 pounds of active ingredient per gallon.)

Dimethoate—50% technical grade wettable powder.

Dipterex®—50% dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate soluble powder.

Di-Syston®—25% *O,O*-diethyl *S*-2-(ethylthio) ethyl phosphorodithioate wettable powder.

Dow ET-14—25% *O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate wettable powder.

Dow ET-15—25% *O*-methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate wettable powder.

Dow M361—25% *O*-methyl *O*-2,4,5-trichlorophenyl N-ethyl phosphoramidothioate wettable powder.

Endrin—10% commercial grade wettable powder.

Ethion—25% commercial grade wettable powder

Guthion®—25% *O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate wettable powder.

Isodrin—10% technical grade wettable powder.

Lead arsenate—Standard spray formulation wettable powder.

Malathion—25% commercial grade wettable powder.

Malathion SF—Emulsifiable concentrate containing 2½ lbs. actual malathion per gallon in a formulation purported to increase residual activity.

Table 2.—Results of complete block orchard tests for apple maggot control, 1954–1960.

Test No.	Materials (lb./100 gal.)	Dates of Applications	Fruit Examined[a]		Injured (%)
			Variety	Number	
		1954			
1	50% Perthane, 2+lead arsenate, 2+lime, 2 50% Perthane, 2	June 22, July 6, 20 Aug. 4, 17	McIntosh	6,634	3.0
			G. Delicious	3,547	2.4
			Total	10,181	
			Average		2.8
			1953 Average		26.6
2	50% Strobane, 2	June 22, July 2, 12, 22, Aug. 4,16	McIntosh and Macoun	255	15.3
			Cortland	308	42.9
			G. Delicious	122	28.7
			Total	685	
			Average		29.0
			1953 Average		59.4
3	25% Diazinon, 2	June 25, July 6, 16, 26, Aug. 5, 16	Delicious	19,759	
			Average		0.1
			1953 Average[b]		75.0±
4	25% Diazinon, 1+50% DDT, 1	June 25, July 6, 16, 26, Aug. 5, 16	Delicious	6,698	
			Average		0.0
			1953 Average[b]		75.0±
		1955			
5[c]	25% Diazinon, 1+50% DDT, 1	June 24, July 6, 15, 25, Aug. 4	McIntosh	11,041	1.2
			Delicious	8,790	4.3
			Total	19,831	
			Average		1.9
			1954 Average[b]		40.0±
6	25% Diazinon, 2	June 22, July 18	McIntosh, Macoun and Cortland	2,152	90.7
			G. Delicious	63	88.9
			Total	2,215	
			Average		89.8
			1954 Average		9.0
7	25% Dow ET-14, 2	June 22, July 1, 11, 21, Aug. 1	Wealthy	9,731	
			Average		83.0
			1954 Average[b]		95.0±
8	25% Dow ET-15, 2	June 22, July 1, 11, 21, Aug. 1	Wealthy	10,369	
			Average		79.1
			1954 Average[b]		95.0±
		1958			
9	25% ethion, 2	June 30, July 10, 21, 31, Aug. 12	McIntosh and Macoun	8,707	38.9
			Cortland	1,944	85.4
			G. Delicious	3,012	87.8
			Total	13,663	
			Average		70.7
			1957 Average[b]		95.0±
10	50% Sevin, 1	June 30, July 10, 21, 31, Aug. 12	Delicious	6,444	
			Average		89.9
			1957 Average[b]		65.0±
11	25% Guthion, 0.75	June 30, July 10, 21, 31, Aug. 12	McIntosh	8,409	
			Average		49.7
			1957 Average[b]		65.0±
		1959			
12	50% Sevin, 2	June 18, July 2, 16, 30, Aug. 13	Early McIntosh	435[d]	5.8
			McIntosh	594	28.3
			Cortland	424	36.3
			Delicious	451[d]	60.6
			G. Delicious	259[d]	66.0
			Total	2,163	
			Average		42.9
			1958 Average[b]		95.0±

(*Continued on next page*)

[a] Total crop from six or more trees, except Tests 7 and 8 which had four counted trees in each.
[b] Estimated.
[c] Applied as an air-blast spray.
[d] Partial crop records, unknown amount lost.

Table 2.—(*Continued*)

Test No.	Materials (lb./100 gal.)	Dates of Applications	Fruit Examined[a]		Injured (%)
			Variety	Number	
13	Phosphamidon 4, 0.5 pt.	June 26, July 6, 16, 27, Aug. 6, 17	McIntosh, Macoun, Cortland, and N. Spy	3,776	72.6
			G. Delicious	739	89.7
			Total	4,515	
			Average		81.1
			1959 Average		70.7
14	50% dimethoate, 2	June 24, July 2, 13, 23, Aug. 4, 13	Wealthy	8,717	5.8
			Delicious	8,566	29.7
			Total	17,283	
			Average		12.8
			1958 Average		89.9
15	50% Bayer 22408, 2	June 24, July 2, 13, 23, Aug. 3, 13	McIntosh	12,065	
			Average		16.9
			1958 Average		49.7
		1960			
16	25% Guthion, 1	June 20, July 5, 18, Aug. 1, 15	Delicious	5,435	
			Average		3.2
			1959 Average		42.9
17	50% Sevin, 1+lead arsenate, 2+ferbam, 0.5	June 28, July 11, 22	McIntosh, Macoun and Cortland	1,767	91.1
	50% Sevin, 1	June 16, Aug. 4, 15	G. Delicious	810	99.4
			Total	2,577	
			Average		95.2
			1959 Average		81.1
18	Malathion SF emulsion, 26 oz.	June 17, 27, July 7, 18, 28, Aug. 8, 18	McIntosh	6,033	9.7
			Jonathan	10,153	74.1
			Total	16,186	
			Average		38.6
			1959 Average[b]		75.0±

Methoxychlor—50% commercial grade wettable powder.
Perthane®—50% 1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane wettable powder.
Phosdrin®—10% 1-methoxycarbonyl-1-propen-2-yldimethyl phosphate (and related compounds) wettable powder.
Phosphamidon—49% commercial liquid concentrate containing 4 lbs. active ingredient per gallon.
Phostex®—25% of a mixture of bis (dialkyloxyphosphinothioyl) disulfides in wettable powder formulation .
Pirazinon®—25% *O,O*-diethyl-*O*-3-(2-propyl-6-methyl-4-pyrimidinyl) phosphorothioate wettable powder.
Ryania—100% wettable powder standard grind and 95% micronized wettable powder.
Sevin®—50% 1-naphthyl *N*-methylcarbamate wettable powder.
Strobane®—50% of a mixture of chlorinated terpenes with 66% chlorine in wettable powder formulation.
TDE—50% commercial grade wettable powder.
Thiodan®—25% 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide wettable powder.
Trithion®—25% *S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate wettable powder.

LABORATORY TESTS.—Results of laboratory tests are summarized in table 1. Chemicals exhibiting a high degree of toxicity to apple maggot adults in these tests included Dow M361, Dow ET-14, Dow ET-15, Diazinon, Pirazinon, Dipterex, endrin, isodrin, ethion, Thiodan, Bayer 25141, phosphamidon, Dibrom, Phosdrin, Guthion and dimethoate. Of these, Dow ET-14, Dow ET-15, Diazinon, Pirazinon, Dipterex, endrin and isodrin showed a very rapid lethal effect, which is often associated with short residual action. Other materials that showed a good to moderate degree of toxicity to the flies were Chlorthion, DDVP, Sevin, Trithion, Di-Syston, Bayer 22408 and Bayer 29493. Phostex and Ryania were the least effective. In the discontinuous exposure tests of Bayer 25141 and Bayer 29493, it was noted that mortality was high in the check cage located next to the test cages in the laboratory rearing room. This suggested that the materials were acting in vapor form, even in nearby cages. Accordingly, glass slides were sprayed with each compound and placed in the tops of test cages in the usual manner, but with cheesecloth screens one-half inch below them to prevent direct contact of flies with the spray films. As shown in table 1, a rapid kill resulted, indicating that the materials did possess marked vapor toxicity.

FIELD TESTS.—Results of field tests of foliar sprays, in which isolated blocks of trees were treated in entirety with one insecticide or combination of materials, are summarized in table 2. Three tests are included in which the experimental blocks were divided into large plats receiving different treatments, but which were of sufficient size to minimize the effects of fly movement between plats and, hence, are essentially the same as isolated blocks. Several chemicals which showed pronounced toxicity to flies in the laboratory failed to give adequate control in the orchard. Included in this group are Dow ET-14, Dow ET-15, ethion and phosphamidon. It is probable that

Table 3.—Results of small-plat orchard tests with air-blast sprayer for apple maggot control, 1955.

Plat No.	Materials (lb./100 gal.)	Dates of Applications	Fruit Examined[a]		Injured (%)
			Variety[b]	Number	
A and B	50% DDT, 2+lead arsenate, 2	June 27, July 8	McIntosh	6,270	4.0
			Cortland	1,931	13.1
	Lead arsenate, 3	July 20, Aug. 2, Aug. 15	Total	8,201	
	50% TDE, 1.5		Average		6.4
	50% methoxychlor, 2				
C	DDT-Diazinon E.C. #455, 1 qt.	June 27, July 8, 20, Aug. 2, 15	McIntosh	2,722	7.6
			Cortland	1,293	29.5
			Total	4,015	
			Average		15.8
D	50% DDT, 1+25% Diazinon, 1	June 27, July 8, 20	McIntosh	4,085	15.4
			Cortland	100	2.0
	50% TDE, 1+25% Diazinon, 1	Aug. 2	Total	4,185	
			Average		13.7
	50% methoxychlor, 1+25% Diazinon, 1	Aug. 15			
E	25% Diazinon, 1+25% malathion, 1	June 27, July 8, 20, Aug. 2, 15	McIntosh	2,384	49.7
			Cortland	865	88.9
			Total	3,249	
			Average		59.5
		Per Cent Infested 1954 (est.)			75.0±

[a] Samples of 300 apples, or less, per tree at each collection.
[b] Eight trees per plat except seven in Plat A, variable number of each variety.

their failure in the field was due to insufficient residual action. Under practical conditions, a minimum protection period of 10 days is considered essential, and one of 12 or 14 days is desirable. Strobane, dimethoate, Bayer 22408 and malathion SF formulation gave partial control but were not considered effective enough for commercial use. In general, their failure can be attributed to a lack of residual action in the orchard since their toxicity was demonstrated in the laboratory. Perthane, which had previously proved effective by itself (Dean 1954), also controlled apple maggot at longer spray intervals when supplemented with lead arsenate during the period of heaviest maggot attack. The effectiveness of Diazinon, either alone or combined with DDT, was amply demonstrated. However, when used only twice during the period of maggot activity, it failed. Applied at 10-day intervals, it was one of the most effective insecticides tested. The results of the 1955 tests with Diazinon combinations (table 3) indicate that, at 12-day intervals between applications, control is reduced, especially from the Diazinon-malathion combination. The value of Sevin for apple maggot control is controversial. One pound of the 50% Sevin wettable powder per 100 gallons, at 10-day intervals, or 2 pounds per 100 gallons, at 12- or 14-day intervals, did not prevent enough fruit-puncturing to give practical control, although larval development within the fruit was curtailed or may even be stopped completely (Garman 1960). The criterion for injury used in this study is puncturing of the fruit by ovipositing flies since this, in itself, is sufficient to cause serious deformation of some apple varieties. On this basis, Sevin must be considered ineffective unless used at the rate of 1 pound of actual material per 100 gallons applied at intervals of not more than 10 days. Dimethoate was found to have a similar effect in that it did not prevent fruit puncturing in excess of commercially acceptable amounts, although larval development did not occur in fruit sprayed at approximately 10-day intervals with 2 pounds of 50% dimethoate per 100 gallons. Guthion provided good control, under field conditions, when used in adequate amounts. Three-quarters of a pound of 25% Guthion per 100 gallons, at 10-day intervals, did not give satisfactory control, but 1 pound per 100 gallons was effective when used at 12- or 14-day intervals.

In table 3, results of a small-plat experiment are given. An unsprayed check plat was not included, hence per cent control efficiencies cannot be calculated, but direct comparison of the different treatments is possible. Control was not satisfactory with the Diazinon-DDT and Diazinon-malathion combinations used, as stated above, possibly because of too long an interval between applications. Inadequate spray coverage may also have contributed, since the method of application used (air-blast machine) is generally regarded as less thorough than hand-spraying. However, the DDT-lead arsenate-TDE-methoxychlor schedules did reduce maggot damage to a considerable extent, although they were applied by the same means, and their success encouraged the use of the experimental design employed in the tests reported in table 4. This table contains data from small-plat tests which included untreated check plats and in which per cent control efficiency values were calculated. The tests were conducted on succeeding years in the same orchard, using the same method of spray application (operator on top of sprayer tank with hand gun or four-nozzle broom), and the same sampling technique in taking harvest records. There was no evidence of significant fly movement from check plats to nearby treated trees, or from less effective treatments to adjoining ones. Nevertheless, trees on which counts were made were selected from inner

Table 4.—Results of small-plat orchard tests for apple maggot control, 1959–1960.

Plat No.	Materials (lb./100 gal.)	Dates of Applications	Fruit Examined[a]		Injured (%)	Control Efficiency (%)
			Variety[b]	Number		
		1959				
1a and 2a	50% Sevin, 2	June 16, 29, July 10,22, Aug. 3, 14	McIntosh	6,007	9.0	
			Cortland	4,922	20.3	
			Total	10,929		
			Average		14.7	81.9
3a	50% DDT, 2	June 16, Aug. 14	McIntosh	2,642	1.4	
	50% DDT, 2+lead arsenate,[c] 2	June 29, July 10, 22	Cortland	2,657	3.1	
	50% TDE, 2	Aug. 3	Total	5,299		
			Average		2.2	97.2
4a	Glyodin,[d] 1.5 pts.	June 16	McIntosh	2,654	67.1	
			Cortland	7,016	87.6	
			Total	9,670		
			Average		80.8	—
		1960				
1, 2 and 3	50% Sevin, 2	June 16, 27, July 8, 20, Aug. 1, 12	McIntosh	5,164	1.8	
			Cortland	5,060	15.3	
			Total	10,224		
			Average		8.5	87.6
5 and 6	50% DDT, 2	June 16, Aug. 12	McIntosh	3,242	0.4	
	50% DDT, 2+lead arsenate,[c] 2	June 27, July 8, 20	Cortland	3,424	7.0	
	50% TDE, 2	Aug. 1	Total	6,666		
			Average		3.7	94.6
7	50% DDT, 2	June 16, Aug. 12	McIntosh	1,677	0.1	
	50% DDT, 2+lead arsenate,[c] 2	June 27, July 20	Cortland	1,466	1.2	
	25% Trithion, 1	July 8	Total	3,143		
	50% TDE, 2	Aug. 1	Average		0.6	99.1
8	50% DDT, 1+25% Trithion, 0.5	June 16, 27, July 8, 20, Aug. 1, 12	McIntosh	1,800	1.9	
			Cortland	1,724	34.2	
			Total	3,524		
			Average		18.0	73.7
9	25% Guthion, 1	June 16, 27, July 8, 20, Aug. 1, 12	McIntosh	1,668	0.1	
			Cortland	1,639	4.1	
			Total	3,307		
			Average		2.1	96.9
10	50% Sevin, 1	June 16, Aug. 12	McIntosh	1,823	0.1	
	50% Sevin, 1+lead arsenate,[c] 2	June 27, July 8, 20	Cortland	1,481	1.4	
	25% Guthion, 1	Aug. 1	Total	3,304		
			Average		0.8	98.9
11	50% Sevin, 0.5+25% Guthion, 0.5	June 16, 27, July 8, 20, Aug. 1, 12	McIntosh	1,722	0.5	
			Cortland	1,547	2.1	
			Total	3,269		
			Average		1.3	98.1
12	25% Guthion, 0.5+50% DDT, 1	June 16, Aug. 12	McIntosh	1,505	0.5	
	25% Guthion, 0.5+lead arsenate,[c] 2	June 27, July 8, 20	Cortland	1,684	0.8	
	25% Guthion, 0.5+50% TDE, 1	Aug. 1	Total	3,189		
			Average		0.6	99.1
4	Control (no spray)		McIntosh	3,387	58.9	
			Cortland	2,725	78.2	
			Total	6,112		
			Average		68.6	—

[a] Samples of 300 apples, or less, per tree at each collection.
[b] Three trees of each variety per plat, except 4a, which included two McIntosh and four Cortland.
[c] Ferbam 0.5 lb. per 100 gallons included as safener.
[d] Included in all 1959 plats.

rows of each plat to avoid possible effects from migration. Fifty per cent Sevin, at 2 pounds per 100 gallons, applied at approximately 12-day intervals, gave fair control of apple maggot, but was inferior to the schedule using DDT, lead arsenate and TDE, the "standard" program, in both 1959 and 1960 tests. Substitution of 25% Trithion, at 1 pound per 100 gallons, for one DDT-lead arsenate application in the "standard" schedule increased control slightly, but a combination of 50% DDT, at 1 pound, and Trithion at 0.5 pound, per 100 gallons in all sprays gave poor control. Guthion (25%) wettable powder 1 pound per 100 gallons, or at 0.5 pound plus 50% Sevin 0.5 pound per 100 gallons, in complete schedules, gave a high degree of control of apple maggot, as did a schedule based on Guthion at 0.5 pound per 100 gallons combined with either 1 pound of DDT, 2 pounds of lead arsenate or 1 pound of TDE. Contrary to the results of the complete block test of Sevin plus lead arsenate (test 17, table 2), a similar program which included a single application of Guthion, at 1 pound per 100 gallons (plat 10, table 4), also yielded satisfactory maggot control.

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Effectiveness of DDT for Cabbage Caterpillar Control in Indiana, 1945 to 1960[1]

GEORGE E. GOULD, *Purdue University, Lafayette, Indiana*

ABSTRACT

Resistance of the imported cabbageworm (*Pieris rapae* (L.)) and the cabbage looper (*Trichoplusia ni* (Hbn.)) to DDT was not apparent in experimental plots until 1958, although growers of commercial acreages of cabbage and related crops complained of lack of control as early as 1953. In 1944 and 1945 tests with DDT against these two caterpillars showed satisfactory results with as little as one-half pound actual toxicant per acre. Trials with DDT and other new insecticides were continued from 1944 through the 1960 season. DDT at a standard rate of 1 pound of actual toxicant per acre was adequate in most seasons up to 1958, although some other insecticides excelled in over-all ratings in some years. In 1958 DDT rated sixth among eight insecticides and so in 1960 the rate per acre was increased to 2 pounds. Again DDT was one of the better materials, although the kill of loopers in the first 24 hours was poor.

Both endrin and toxaphene gave consistently good control when included in the tests. Dieldrin was effective in some years and results were mediocre in others. In general the phosphate insecticides have been inferior to the chloronated hydrocarbon materials. They often gave a good quick kill, but in some instances plants were reinfested within a week. Two new materials, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) and Sevin® (1-naphthyl *N*-methylcarbamate), showed promise but have not been tested sufficiently for accurate evaluation. The biological pest control agent made up of live spores of the microorganism, *Bacillus thuringiensis* Berl., gave a good initial kill of the caterpillars, but permitted a build-up of the population within a week.

Two insects, the imported cabbageworm (*Pieris rapae* (L.)) and the cabbage looper (*Trichoplusia ni* (Hbn.)), continue to be a major problem in Indiana in the production of cabbage and related crops, even though many insecticides have been tested and recommended for control. When DDT first became available and was tested in 1945 and 1946, this new insecticide gave spectacular control of both caterpillars. Then in 1953 and 1954 several commercial growers in Marion and Lake counties, Indiana, reported poor results with DDT, although in experimental plots near Lafayette, DDT at the standard rate of 1 pound actual toxicant per acre continued to be one of the better materials until the 1958 season. Thus this recent development of resistance to DDT by the two caterpillars has made necessary a re-evaluation of control methods in order to make recommendations to growers.

A review of the literature shows that the first indication of a DDT-resistant strain of imported cabbageworms was in 1951 in southeastern Wisconsin (McEwen & Chapman 1952). Several other states soon reported lack of control with DDT, while in 1955 DDT resistance in the cabbage looper was noted in western New York (McEwen & Hervey 1956) and in Wisconsin (Chapman 1956). Within 2 years most eastern and midwestern states had observed this failure of DDT to control the two caterpillars, although some related compounds, such as endrin and toxaphene, were still effective.

In Indiana the imported cabbageworm is the most important pest of cruciferous crops, although the cabbage looper is usually present and in some seasons is the more destructive of the two. The diamondback moth (*Plutella maculipennis* (Curt.)) is often present, but the larvae are seldom abundant enough to be noticeable. In Purdue tests

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the looper has been more difficult to kill than the cabbageworm, even in the days when arsenicals were used (Gould 1938). Some of this difference can be explained by the feeding habits of the two species. The cabbageworm feeds around the head and on the upper surface of the foliage, while the looper feeds on the lower surface of leaves and usually the lower leaves. In Indiana growers can usually produce an early crop of cabbage with little or no trouble from caterpillars, but they always have to protect the summer and fall crops with insecticides. Since loopers continue to feed through most of September, late applications, even after head formation has started, usually have been necessary.

METHODS AND MATERIALS.—The experimental plots were located on one of the three experimental farms near Lafayette 60 miles or more from commercial cabbage fields. Cabbage plants were used in all seasons and were set in the field around July 1. The first applications were made when insect populations became destructive, usually by August 1, and later applications were made as needed. Three to five applications were made in most seasons. Sprays were applied with a knapsack sprayer at a rate of 125 to 150 gallons per acre, while dusts were applied with a hand duster at a rate of 40 to 50 pounds. Plots were randomized and were usually replicated five times.

The effectiveness of the different materials in checking the two species was determined by several criteria. The best criterion was the yield of cabbage, as this would be of prime concern to the grower. However, it was noted that in some seasons with a late freeze, plants in the untreated and inferior treatments did recover somewhat in late September and October and produced a saleable head. Other criteria used were the number of live and dead worms and loopers present on or under plants before and after treatments, and the amount of feeding to foliage and heads. For evaluating feeding damage two people, working independently, assigned a rating to each plant. Plants with no damage were rated 9, those with little feeding 8 or 7, and on down to 3, 2 or 1 for those with serious feeding injury. Such foliage damage ratings were taken at various times so as to obtain information at application time and 7 and 14 days later. The changes in these figures were indicative of the improvement in plant growth after treatments and of the persistence of residues.

RESULTS.—In our first trials in 1944 and 1945 (Gould 1945, 1946), DDT used as a 3% dust and as a spray at one-half pound per acre gave excellent control of both species. Retreatment was not necessary for 21 days. On the adjacent rows treated with the recommended material, a 0.75% rotenone dust, plants were reinfested in 7 days. In 1947 six insecticides were tested. The effectiveness of the materials was best shown by the number of caterpillars present 10 days after the August 30 applications. The number of insects on 20 plants were as shown in the following table.

TREATMENTS	*Trichoplusia ni*	*Pieris rapae*
DDT 3% dust	0	1
Toxaphene 2% dust	1	0
Parathion 1% dust	1	0
TDE 3% dust	3	0
Parathion spray (0.4 lb. per acre)	3	0
Parathion 0.25% dust	6	2
BHC dust (1% gamma)	15	10
Chlordane 2% dust	36	30
Untreated	112	61

In 1950, the tests included seven dust mixtures and five sprays. The sprays included DDT alone and combined with lindane, and three new phosphate insecticides, demeton, schradan and Potasan® (4-methylumbelliferone *O,O*-diethyl thiophosphate). DDT alone and with lindane in spray formulations and DDT combined with rotenone as a dust gave good control. The three phosphate compounds gave poor control at the dilutions used in these tests.

The 1952 trials included for the first time three new insecticides, endrin, Perthane® (1,1-dichloro-2,2-bis (*p*-ethylphenyl) ethane) and EPN. Late cabbage was treated with 5 sprays and 2 dust mixtures on 2 dates, August 20 and September 12. The efficiency of the treatments, based on the foliage damage rating and the yield at harvest, showed endrin was best, closely followed by DDT and Perthane. The dust and spray mixtures containing methoxychlor, rotenone and pyrethrins gave poor protection, although the yields were considerably higher than the untreated plots.

In 1953, the cabbageworm was the principal pest with only a few loopers present. Four materials were included in the tests and were applied on three dates: July 27, August 12 and 31. Two criteria were used in evaluating results: the number of dead and live worms found 2 days after the first application and foliage damage rating 7 days after the third application. These results were as follows:

TREATMENT	*Pieris rapae* Dead	*Pieris rapae* Alive	DAMAGE RATING
Strobane®[a] spray, 2 lb. actual/A.	9	28	8.5
Perthane spray, 1 lb. actual/A.	21	44	6.5
Malathion 4% dust	38	0	6.5
Methoxychlor 5%-rotenone 1% dust	19	4	4.7
Untreated	0	120	2.0

[a] Terpene polychlorinates (66% chlorine).

Reports of the failure of DDT to control cabbageworms satisfactorily were received from Lake and Marion counties, Indiana, in 1953 and 1954. In our experimental plots, DDT at 1 pound actual toxicant per acre was still among the better materials and was giving protection for 10 to 14 days. In 1954, DDT was compared with several other chlorinated hydrocarbon insecticides as well as with two phosphates, malathion and Chlorthion® (*O,O*-dimethyl *O*-(3-chloro-4-nitrophenyl) thiophosphate). During that season both loopers and worms were abundant and three applications, July 28, August 6 and 24, were necessary to hold them in check. The larval population built up again in September, but no additional application was made because heads were forming. Ratings of foliage damage were made on seven dates, which have been combined with the three shown in table 1. The August 11 data are an average of the first three ratings, the August 27 data

Table 1.—Effectiveness of seven insecticides for the control of cabbageworms and loopers as determined by cabbage foliage ratings and the yield. 1954.

Insecticide	Toxicant per Acre (lb.)	Foliage Damage Rating Aug. 11	Aug. 28	Sept. 13	Cabbage per Plot (lb.)
Endrin 1.5E	0.3	8.2	8.3	8.5	29.6
Dieldrin 1.5E	0.3	8.9	7.8	8.2	28.5
DDT 2E	1.0	8.3	7.6	7.9	27.7
Strobane 8E	2.0	8.9	8.4	8.6	24.6
Toxaphene 6E	2.0	7.9	6.9	8.1	24.6
Malathion 5E	1.3	7.9	7.3	7.0	18.5
Chlorthion 4E	1.0	7.6	5.2	5.1	10.6
Untreated	—	2.5	3.5	3.9	2.9
L.S.D. 19:1		1.4	0.8	0.6	4.3
99:1		1.9	1.0	0.8	5.8

Table 2.—Effectiveness of ten insecticides for the control of cabbageworms and loopers as determined by foliage ratings and the percentage of worms dead 24 hours after application, and the yield of cabbage. 1955.

Insecticide	Toxicant per Acre (lb.)	Average Foliage Damage Rating	Worms Dead After 24 Hours (%)	Cabbage per Plot (lb.)
Toxaphene 6E	2.0	7.5	67	23.1
Endrin 1.5E	0.3	7.3	80	22.9
Strobane 8E	2.0	7.0	55	21.9
Malathion 5E	1.3	7.0	93	21.2
Dieldrin 1.5E	0.3	7.0	90	19.9
Heptachlor 2E	0.3	6.7	55	19.2
DDT 2E	1.0	7.3	83	18.9
Methoxychlor 2E	1.0	7.0	83	16.7
Pyrenone 606[a]	*	7.1	95	13.8
Chlorthion 4E	1.0	6.8	52	13.4
Untreated	—	5.8	0	8.5
L.S.D. 19:1		0.6	—	4.8
99:1		0.8	—	6.4

[a] Commercial preparation, manufacturer's directions followed.

are an average of the third and fourth ratings, and the September 13 data are an average of the last two ratings. The high ratings throughout the season for endrin and Strobane indicated a long residual action for these two materials.

In 1955, both the cabbageworm and the looper were again abundant and did considerable feeding until mid-September when the populations decreased rapidly. Since the plants were set late, most of them produced satisfactory heads by November when a heavy freeze stopped further growth. DDT used at 1 pound actual toxicant per acre was still giving satisfactory control, although the yield was somewhat lower than the most effective material, toxaphene. Excellent kills in the first 24 hours after treatment were observed for dieldrin, malathion and a pyrenone formulation, while endrin, DDT, and methoxychlor killed 80% or more. Results of these tests are given in table 2.

The looper and worm populations were again high in 1956. Four new insecticides, Sevin® (1-naphthyl N-methylcarbamate) and three phosphates, Guthion® (*O,O*-dimethyl S-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate), Dylox® (dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate), and ronnel, were included in the tests. Guthion gave excellent control, but was not significantly better than DDT and Sevin. The yield from the Dylox-treated plots was among the better treatments, but in foliage protection its rating was low. On the other hand ronnel gave good foliage protection, but the weight of cabbage harvested was low.

In 1957 six materials were under test and were applied on three dates: August 7, 23 and 29. The efficiency of the different formulations was based on yield and the foliage damage ratings made on August 14, 23, 29, and September 6. In 1957, the best protection was given by a "one-package" garden dust containing 4% malathion and 4% methoxychlor. The foliage damage rating of this dust was 7.8, for DDT spray at the 1-pound rate 7.3, heptachlor at 0.5 pound 7.0, and dieldrin at 0.3 pound 7.0. Both Sevin and a new phosphate insecticide, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thiophosphate) gave

Table 3.—Effectiveness of eight insecticides for the control of cabbageworms and loopers as determined by cabbage foliage ratings and yields. 1958.

Insecticide	Toxicant per Acre (lb.)	Average Foliage Damage Rating	Cabbage per Plot (lb.)
Endrin 1.5E	0.3	8.3	26.6
SD-4402 1.25E	0.3	8.4	23.9
Kepone 2E	1.0	7.8	20.1
Sevin 2E	1.0	6.8	19.9
Dieldrin 1.5E	0.3	7.5	18.8
DDT 2E	1.0	7.4	18.8
Heptachlor 2E	0.5	6.6	18.5
Diazinon 2E	0.5	7.5	14.7
Untreated	—	5.5	7.0
L.S.D. 19:1		0.3	2.7
99:1		0.4	3.7

Table 4.—Imported cabbageworm and cabbage looper counts and cabbage foliage ratings and yields following various insecticide treatments. 1960.

Insecticide	Toxicant per Acre (lb.)	Live Loopers per Plant on Aug. 30	Sept. 10	Foliage Damage Rating on August 10	18	25	Cabbage per Plot (lb.)
Toxaphene 6E	3.0	0.	5.1	8.5	8.1	8.4	40.1
Endrin 1.6E	0.3	2.0	1.4	8.5	8.4	8.7	39.7
DDT 2E	2.0	11.0	5.2	8.1	7.9	8.3	37.6
Thiodan 2E	0.75	4.0	5.0	8.0	8.0	8.6	36.8
Toxaphene 6E-malathion 5E	2.0 2.0	7.0	4.4	8.0	7.8	8.3	35.3
SD-4402 1.25E	0.15	6.0	6.0	7.9	6.8	8.0	34.1
Malathion 5E	2.5	23.0	10.4	7.9	7.0	7.7	33.6
SD-4402 1.25E	0.3	4.0	7.0	8.0	7.3	8.1	33.1
Dibrom 8E	1.0	17.0	8.5	7.7	6.7	8.0	32.3
Thuricide	2.0	16.0[a]	5.0	7.7	7.0	7.9	31.9
Thuricide	4.0	14.0	3.5	7.7	6.9	7.9	31.7
Perthane 4E-malathion 4E	1.0 1.25	5.0	9.6	8.0	7.7	8.2	31.6
Dimethoate 4E	1.0	22.0	4.9	7.9	7.3	7.9	31.2
Perthane 2E	2.0	29.0	9.4	8.0	7.6	7.6	30.0
Diazinon 2E	0.5	21.0	6.8	7.5	6.0	7.3	29.1
Bayer-29493 4SC	1.0	13.0	7.7	7.9	6.9	8.0	28.0
Untreated	—	8.0[a]	11.4[a]	3.5	4.1	6.0	16.0
L.S.D. 19:1		—	—	0.5	0.5	0.3	4.6
99:1		—	—	0.7	0.7	0.4	6.1

[a] In addition, Thuricide had an average of 3 cabbageworms per plant on the first date, while the untreated rows had an average of 8 worms per plant on the first date and 5.7 on the second.

relatively poor protection to the foliage, but rated high in yield of cabbage.

In 1958 the populations of both the cabbageworm and the looper were again high. Included in the eight materials in the tests were two new insecticides, Kepone® (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta (cd) pentalen-2-one) and Shell Dev. 4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan). All materials were used as sprays and were applied on August 13, 22 and September 8. Damage to foliage was evaluated on five dates from August 21 to September 25 and the average of the five evaluations is given in table 3. In these tests DDT at 1 pound actual per acre was definitely inferior to several of the other materials. As most caterpillars had disappeared by mid-September and most heads harvested by mid-October, the yields were high.

Because of the failure of DDT at the 1-pound rate to give good protection in 1958 and in some other seasons, the rate used for this insecticide in 1960 was increased to 2 pounds of the actual toxicant per acre. Also included were several materials that had shown promising results in previous years plus several new compounds. Among the new insecticides were Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), dimethoate, and Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate). Another new material was Thuricide®, a biological pest control agent whose active principal is live spores of the microorganism, *Bacillus thuringiensis* Berliner. This formulation contained 30 billion viable spores per gram. Parrott (1960) tested Thuricide in 1959 for the control of cabbageworms and loopers and found that the material gave good control of the worms only when applications were repeated at about 6-day intervals. The rate of application was 3 pounds per acre. The author also used this material against the mint looper, *Rachiplusia ou* (Gn.), on peppermint and spearmint in 1959 and 1960. The mint looper population was low in both years, but those present were not appreciably checked by Thuricide applied as a spray at 2 or at 4 pounds per acre.

In 1960 the cabbageworm and the looper were both abundant on the cabbage plots in August, but the worm population decreased rapidly in early September. Spray applications were made July 29, August 17 and 30. A spreader, Triton B-1956, was added to all sprays to increase spreading and sticking of the insecticide to the foliage. The first application of several of the sprays caused a slight burning of the foliage, while all applications of an experimental material, Bayer 29493 (*O,O*-dimethyl *O*-(4-(methylthio)-*m*-tolyl) phosphorothioate), caused some injury. Loopers continued abundant through September, although a fungus disease, as yet unidentified, killed many on both the treated and the untreated rows. Leaves on untreated plants started to grow out in late September and many plants produced small heads.

The efficiency of the various treatments as determined by the numbers of live loopers and worms present, the rating of foliage damage, and the yield of cabbage, is given in table 4. To determine the number of caterpillars present on August 30 and September 10, 25 to 40 plants from each treatment were carefully dissected. Only three treatments, Thuricide at the 2-pound rate on the first date and the untreated on both dates, had any worms. Loopers were rather abundant, with a high of 29 per plant on the Perthane-treated plots. DDT used at the increased rate of 2 pounds was one of the best, while endrin and toxaphene rated at the top. Thiodan was also one of the better materials. Thuricide gave a poor kill of loopers and was only slightly better on cabbageworms.

DISCUSSION.—In experimental plots DDT remained one of the better materials for controlling the cabbageworms and loopers through the 1957 season, although growers of commercial crops complained of lack of control as early as 1953. Endrin at 0.3 pound and toxaphene at 2 pounds were the best materials, although in 1960 the dosage rate of the latter material was increased to 3 pounds. The phosphate insecticides were inferior, in most comparisons, to the chlorinated hydrocarbon materials. The phosphate group often gave a good initial kill, but plants were reinfested within 7 to 10 days.

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Control of the Angoumois Grain Moth, *Sitotroga cerealella*, in Stored Earcorn with Malathion in Illinois, 1959–1960[1]

STEVE MOORE, III and G. C. DECKER, *University of Illinois and Illinois Natural History Survey, Urbana*

ABSTRACT

Farm storages of earcorn were treated at various times with dusts and sprays of premium grade malathion to protect against damage by the Angoumois grain moth, *Sitotroga cerealella* (Oliv.). Various dosages and two different dust formulations of premium grade malathion were tested. Untreated cribs of earcorn were utilized for comparative purposes. The tests were conducted over the spring and summer months in four counties located in the area of southern Illinois threatened by the Angoumois grain moth.

All treatments tested provided good to excellent control of the Angoumois grain moth from April through August. An 89.4% average reduction in damage was obtained for all treatments during the two seasons of tests. This amounted to a savings due to treatment each year of 952 pounds, or 17 bushels, in each 1000 bushels of earcorn under storage. Eleven of the twelve cribs of untreated earcorn dropped one or more grades due to damage by the Angoumois grain moth while not a single treated crib of earcorn dropped in grade. The numbers of other stored-grain insect pests were reduced 80% by these treatments.

It was suggested that a single application of a spray to the surface and sides of the earcorn in April or a single dust treatment applied at the time the corn is cribbed in the fall is the most practical method of application. Treatments of this type would adequately protect stored earcorn in southern Illinois from damage by the Angoumois grain moth for approximately a full season. This would be an advantage to any farmer storing earcorn beyond the middle of June in this area.

Farmers who have earcorn in storage in the southern one-third of Illinois between May and October suffer losses from the Angoumois grain moth. Even farmers storing their earcorn until late June and July under the C.C.C. (Commodity Credit Corporation) loan and purchase agreement program often experience economic loss due to this insect. In general, the Commodity Credit Corporation prefers a delayed "take-over" of their corn from farms to allow for sufficient drying for safe storage in the shelled form. Conversely, the farmers prefer an early "take-over" to avoid damage from the Angoumois grain moth since the farmers must deliver to Commodity Credit Corporation the same grade of corn called for in the original purchase agreement.

Both the farmers and C.C.C. would benefit if a convenient, effective, and inexpensive full season control was developed for the Angoumois grain moth. Presently, no practical and effective means of controlling this insect is available to farmers. Malathion was selected for use in this study because of its low toxicity, relatively low cost, and promising performance against other stored-grain insects. The first report on the use of malathion against insects infesting stored grains was published by Lindgren *et al.* in 1954. Since that time many other workers have reported on the effectiveness of malathion against stored grain insect pests. Moore (1959) presented 3 years' data showing that malathion was highly effective for a full season when used as a dust or spray at harvest time to control insects attacking stored wheat. It was the purpose of this study to determine the effectiveness of various dosages, formulations, methods of application and times of application of malathion for the control of the Angoumois grain moth in stored earcorn.

MATERIALS AND METHODS.—Fourteen cribs of earcorn located on seven different farms in four counties in the southern tip of Illinois were utilized. All cribs were of the wooden slat type construction except two which were constructed with wire sides. A check and a treated crib, commonly of the double style, were present on each farm for comparative purposes. Each crib contained from 400 to 4000 bushels of earcorn.

During the latter part of April, 1959, five cribs of 1958 earcorn were treated with premium grade malathion as a dust and spray (table 1). The surface of the earcorn was treated with the dust form, consisting of 1.0% premium grade malathion on wheat flour. The dust was scattered by hand at the rate of 20 to 50 pounds per crib, depending on the surface area. The sides of the cribs and exposed earcorn were sprayed to run-off with a 2.75% water diluted emulsion spray of premium grade malathion. The spray was applied from a 3-gallon tank sprayer with a 5-foot spray rod at 50 to 60 p.s.i. The sides of the Rister and Bremer cribs (table 1) were retreated in June with a spray treatment similar to that applied in April.

In November, 1959, five cribs of 1959 earcorn were treated with either 2% or 4% premium grade malathion on corn cob flour at the rate of 50 pounds per 1000 bushels. The dust was applied in layers, usually after each wagon load of earcorn was cribbed. In April, 1960, two of these same cribs (Bremer and Moye) and two additional untreated cribs received a top and a side spray of 1.5% premium grade malathion applied to run-off (table 2).

Quart samples were taken at periodic intervals from both treated and untreated cribs with a 5-foot Iowa Earcorn Test Probe, which removes kernels from the ears as it is rotated 180 degrees.[2] The first samples were drawn either in the latter part of November or April, and the final samples were drawn in the latter part of August. The samples were screened to determine the abundance of stored-grain insects other than the Angoumois grain moth, and a moisture reading was obtained. A 100-gram sub-sample was extracted from each quart sample and the kernels showing visible Angoumois grain moth injury were counted and weighed. An estimate of the number of adult moths present on the surface of the earcorn in each crib was made at each observation.

RESULTS.—April treatments of premium grade mala-

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[2] Manufactured by the Seedburo Equipment Company, Chicago, Illinois.

Table 1.—Effect of spring applications of sprays and dusts of premium grade malathion to 1958 earcorn against the Angoumois grain moth. Gallatin, Johnson, Massac, and Union Counties, Illinois, 1959.

Name and County	Dosage in P.P.M.				No. of Adults on Surface			Damaged Kernels in 100-Gram Sample (gm.)			
	April		June								
	Dust on Top	Side Spray	Side Spray	Total	June	July	August	April	June	July	August
Oliver-Johnson	5.1	19.1	None	24.2	0	0	0	1.0	0.3	0.2	2.0
Oliver-Johnson		Untreated			5	0	15	—	0.9	0.8	5.1
Mathis-Massac	8.4	21.0	None	29.4	0	0	5	0.8	0.2	0.0	1.2
Mathis-Massac		Untreated			15	75	75	—	1.2	3.8	8.6
Gurley-Union	6.4	9.9	None	16.3	0	0	0	0.6	0.9	0.9	1.3
Gurley-Union		Untreated			15	35	500	—	2.4	2.7	10.1
Rister-Gallatin	4.8	11.9	11.9	28.6	1	0	15	0.2	0.5	0.0	0.9
Rister-Gallatin		Untreated			3	0	75	—	0.4	0.6	3.4
Bremer-Massac	10.2	25.5	25.5	61.2	3	0	0	0.3	0.0	0.3	4.2
Bremer-Massac		Untreated			—	—	150	—	—	—	16.4

thion applied as a surface dust and a side spray at dosages varying from 16 to 61 p.p.m. gave good control of the Angoumois grain moth in stored earcorn from April through August of 1959 (table 1). An additional side spray applied 2 months later did not improve the level of control. The weight of damaged kernels per 100-gram sample averaged 8.92 grams in the untreated cribs and 2.03 grams in the treated cribs (table 3). Thus, the weight of the damaged kernels in each 100 grams of corn was 6.89 grams more in the untreated cribs than in the treated cribs. Therefore, there was a 78% average reduction in damage due to treatment. There were significantly fewer adult Angoumois grain moths present in the treated earcorn than in the untreated earcorn (table 1). The corn moisture, which ranged between 13.38% and 15.76% during the test period, did not have any apparent effect on control.

November dust treatments applied in layers as the earcorn was cribbed and April top and side sprays of premium grade malathion at dosages varying from 5 to 32 p.p.m. provided excellent control of the Angoumois grain moth from April through August of 1960 (table 2). An additional top and side spray applied in April to the November dust treated earcorn did not increase control.

Table 2.—Effect of fall and spring applications of dusts and sprays of premium grade malathion to 1959 earcorn against the Angoumois grain moth. Gallatin, Johnson, Massac, and Union Counties, Illinois, 1959–1960.

Name and County	Dosage in P.P.M.			No. of Adults on Surface				Damaged Kernels per 100-Gram Sample (gm.)				
	Sept. or Oct.	April										
	Dust Throughout	Top and Side Spray	Total	Nov.	April	July	Aug.	Nov.	April	July	Aug.	August minus Nov.
Gurley-Union	28.6	None	28.6	0	0	0	75	4.5[a]	1.0	3.5	2.9	0
Gurley-Union		Untreated		0	0	100	500	0.7	1.2	2.4	5.3	4.6
Mathis-Massac	28.6	None	28.6	0	0	15	100	6.3[a]	5.6	3.6	4.5	0
Mathis-Massac		Untreated		0	0	100	300	0.5	0.5	5.7	28.1	27.6
Oliver-Johnson	14.3	None	14.3	0	5	5	40	1.4	1.1	0.5	0.8	0
Oliver-Johnson		Untreated		2	15	100	500	1.5	2.1	2.0	5.8	4.3
Bremer-Massac	14.3	4.2	18.5	0	0	0	75	0.8	1.6	2.7	1.3	0.5
Bremer-Massac		Untreated		0	0	200	300	0.5	0.4	2.4	7.8	7.3
Moye-Gallatin	28.6	3.6	32.2	0	0	0	15	0.9	0.4	0.3	0.5	0
Moye-Gallatin		Untreated		0	0	40	100	0.6	0.3	4.8	13.2	12.6
McGuire-Gallatin	None	14.3	14.3	0	0	0	0	0.2	0.7	0.0	0.3	0
McGuire-Gallatin		Untreated		0	0	5	500	0.9	0.7	1.8	2.2	1.3
Rister-Gallatin	None	5.0	5.0	0	0	0	75	0	0	0	0.6	0.6
Rister-Gallatin		Untreated		0	0	5	500	0.2	0.2	0.4	5.7	5.5

[a] Abnormally high field infestation.

Table 3.—Summary of the control of the Angoumois grain moth in stored earcorn with premium grade malathion in Illinois in 1959 and 1960.

Treatment	No. of Cribs		Average No. Grams of Damaged Kernels per 100-Gram Sample in August			No. Other Live Insects Screened from Four 1-Qt. Samples		
	Check	Treated	Untreated	Treated	Reduction (%)	Untreated	Treated	Reduction (%)
Spring 1959								
Top dust and side spray	3	3	7.93	1.50	81.1	14	2	85.7
April top dust and side spray+June side spray	2	2	9.90	2.55	74.2	8	1	87.5
Average			8.92	2.03	78.0			
Fall 1959 and Spring 1960								
Dust throughout	3	3	12.17	0	100	85	3	96.5
Fall dust throughout+April top and side spray	2	2	9.95	0.25	97.5	23	8	65.2
April top and side spray	2	2	3.40	0.30	91.2	44	20	54.6
Average			8.51	0.18	96.0			
Average 1959 and 1960 Tests			8.67	0.92	89.4	34.8	6.8	80.5

The weight of damaged kernels per 100 gram sample averaged 8.51 grams in the untreated cribs and 0.18 gram in the treated cribs (table 3). Thus, the weight of the damaged kernels in each 100 grams of corn was 8.33 grams more in the untreated cribs than in the treated cribs. Therefore, there was a 96% average reduction in damage due to treatment. There were significantly fewer adult Angoumois grain moths present in the treated earcorn than in the untreated earcorn (table 2). The corn moisture which ranged between 12.41% and 18.64% during the test period did not have any apparent effect on control.

Samples from untreated earcorn yielded 140 more live grain insects, excluding the Angoumois grain moth, than a like number of samples from treated earcorn (table 3). This number amounted to a reduction of 80% of the population of other stored grain insect pests due to treatment. Only those insects of primary importance as pests of stored grains were included in these counts. Eleven primary species of stored-grain insects, six species associated with damp grain and two parasitic species, were screened from these samples. The rice weevil, *Sitophilus oryzae* (L.), the only other internal feeder present, was found only in samples of untreated earcorn. The two species of grain insect parasites were present in samples from both treated and untreated earcorn.

DISCUSSION.—In these tests an average of 8.67%, by weight, of the kernels of untreated earcorn stored in the southern one-third of Illinois were damaged by the Angoumois grain moth from April through August of 1959 and 1960 (table 3). The figures for the individual years were nearly identical, being 8.92% in 1959 and 8.19% in 1960. However, southern Illinois has experienced severe winters for the past 3 years, 1957–60, and it is suspected that the level of damage caused by the Angoumois grain moth during the summers of 1959 and 1960 was below average. The over-all reduction in damage for all treatments during the 2 years of tests was 89.4% (table 3). The weight of the damaged kernels per 100-gram sample averaged 8.67 grams for all untreated cribs and 0.92 gram for all treated cribs. Thus, the weight of the damaged kernels in each 100 grams of corn was 7.75 grams more in the untreated cribs than in the treated cribs. Therefore, 77.5 pounds of shelled corn per 1000 pounds, or 4337 pounds in 56,000 pounds (1000 bu. No. 2 shelled corn), were damaged by the moth in untreated cribs. The average loss per kernel of yellow dent corn due to the Angoumois grain moth has been established at approximately 18% (Back 1929, Quinlan 1957). Therefore, 952 pounds, or 17 bushels, per 1000 bushels of No. 2 shelled corn were completely consumed by the moth in the untreated earcorn. Actually, the economic loss would be even greater if the corn were sold on the open market, since in addition to the substantial weight loss, the corn would also be down-graded. Even in early July, before the C.C.C. take-over program was completed in these counties, one untreated crib had dropped from No. 2 to No. 3 grade and another untreated crib of corn was borderline. By the end of August the untreated corn had dropped to No. 3 grade in five cribs, No. 4 grade in two cribs, No. 5 grade in two cribs, and sample grade in two cribs, while only one crib remained at the original No. 2 grade level. In contrast, not a single crib of treated corn had dropped from the No. 2 grade level by the end of August.

Since all treatments tested performed well, it is suggested that a single application of a spray to the surface and sides of the earcorn in April or a single dust treatment applied at the time the corn is cribbed in the fall is the most practical method of control. Actually, the level of control may be increased under normal conditions of usage over that recorded in these tests due to more complete treatments. On most of the test farms the treated cribs were located within 5 to 10 yards of the untreated storages, which probably resulted in abnormal Angoumois grain moth population pressures on the treatment. Treatments of this type would adequately protect stored earcorn in southern Illinois from damage by the Angoumois grain moth for approximately a full season. The treatment would also serve to prevent build-up and subsequent damage to earcorn by other common stored-grain insects. This would be an advantage to any farmer storing earcorn beyond the middle of June in this area.

The April application would either coincide with the emergence of overwintering moths or just shortly precede emergence. It is suspected that the moths and newly hatched larvae are killed primarily by contact action. Live larvae and pupae were consistently found inside kernels taken from treated earcorn, indicating little if any effect from fumigant action of the malathion. There is still the possibility that the greatest effect of malathion in reducing grain insect infestations in stored grains is by a repellency action.

This study is being continued with emphasis on April perimeter spray treatments, lowest effective dosages, formulations, and residues.

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The Dispersion of Field Bees on Alfalfa in Relation to a Neighboring Apiary[1]

M. D. Levin, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2]

Abstract

Data collected by means of marked honey bees did not support the hypothesis that the dispersion of foragers is more uniform in a direction away from a neighboring apiary than it is approaching the same apiary. Bloom attractiveness was a stronger factor in determining the distribution of the marked bees than was the competition from the other apiary.

An experiment reported earlier (Levin 1959) indicated that foraging honey bees tend to work close to their hives. Subsequently, a test was undertaken to determine if competing bees from a neighboring apiary could be considered responsible for this tendency. MacVicar *et al.* (1952) and Braun *et al.* (1953) had shown that the population density of foraging bees in red clover was affected by the distance of the nearest apiary. Levin *et al.* (1960) have indicated that the same phenomenon can be found on alfalfa fields under certain conditions. The primary objective of the experiment herein described was to test the hypothesis that honey bees tend to disperse more uniformly in a direction away from nearby colonies than they do when working towards them.

Methods.—The experiment was conducted in two alfalfa-seed fields oriented in a north-south direction in a narrow valley at Howell, 50 miles west of Logan, Utah. The area south of the fields was sagebrush desert in which there were no commercial apiaries. A commercial apiary of 96 colonies was located between the two seed fields, and the nearest other apiary of 85 colonies was 0.5 mile north (fig. 1).

On July 16 ten cordovan colonies were placed adjacent to the apiary between the fields. As the bees from this location worked toward the south, they were going away from all colonies and approaching the edge of the cultivated land. As they worked northward, they approached the neighboring group of 85 colonies.

Three sampling stations were established at various distances in each direction. Data were collected on July 17, 18, and 19. Rapidly fading bloom did not warrant continuation of the experiment beyond July 19.

Table 1.—Average numbers of honey bees per square yard collected in two directions at different distances for 3 days after cordovan colonies were placed between two alfalfa-seed fields.

Distance (miles)	All Bees		Cordovan Bees	
	North	South	North	South
0.1	2.53	0.79	0.329	0.086
0.3	1.32	0.63	0.130	0.031
0.4	2.02	—	0.123	—
0.5	—	1.31	—	0.024
Days				
July 17	2.15	0.97	0.118	0.024
18	1.99	1.06	0.258	0.055
19	1.75	0.71	0.207	0.062

Four observers each collected samples of 25 to 50 foraging bees with a sweep net at each station each day. The samples were examined to determine what percentages were from the marked colonies. The bees in 20 square-yard areas at each distance were also counted by the same observers. From the percentages of marked bees and the population densities thus measured, the number of cordovan bees per square yard was determined.

Results.—As shown in table 1, there was a gradient in the distribution of marked bees in both directions from the apiary. Although more than four times as many cordovans were working on the field north of the marked colonies as were to the south, as the distance increased the decline was more pronounced to the south. On both fields the sharpest decrease came between 0.1 and 0.3 mile.

North of the colonies the average number of cordovans

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[2] In cooperation with the Utah Agricultural Experiment Station.

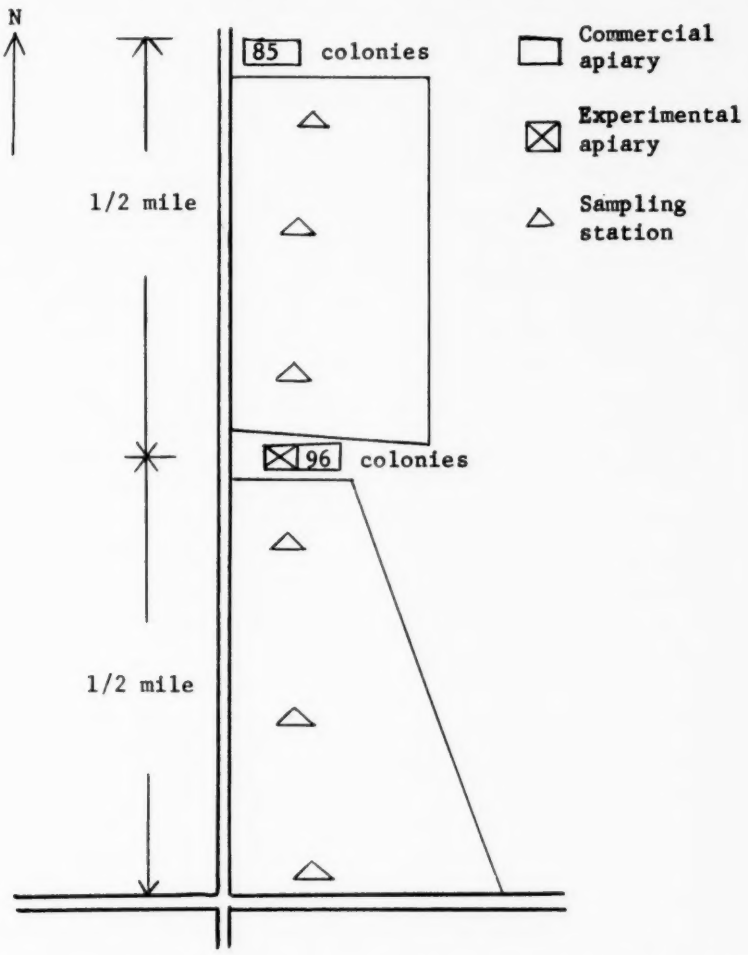

FIG. 1.—Diagram showing experimental alfalfa-seed fields, sampling stations, and apiaries.

collected reached a peak on the second day, but on the field to the south it increased somewhat all 3 days.

The average population between the two apiaries was twice as high as that south of the marked bees. To the north it was at its peak closest to the experimental apiary, but to the south it was highest at the sampling station most distant from the colonies. The average number of bees per square yard decreased at the middle station on the north field, with a rise close to the other apiary.

The mean nectar concentration values at the different stations ranged between 41.1% and 47.3%, showing no particular relationship to distance or direction. The mean soil surface temperatures at a depth of one-half inch were between 101.1° and 108.7° F. They were slightly higher to the north. The mean relative humidity values 6 inches above the ground ranged between 10.6% and 15.5% and were lower on the field to the north.

DISCUSSION.—The results of this experiment indicate that the marked bees dispersed in the field to the south away from the competing apiary in much the same way, although at a considerably lower population level, as they did in the field to the north, where they were working toward another apiary.

Although the data obtained seemed to disprove the hypothesis that bees disperse uniformly away from all colonies and do not when working toward another apiary, the bloom at the various stations proved to be more variable than was expected and probably the application of the findings must be limited. Here, at least, bloom attractiveness was a stronger factor in determining foraging distribution than competition from another apiary.

The effect of the neighboring apiary appears to be shown by the total population data. In the field to the north, bees were more abundant adjacent to both apiaries than at the station midway between. Towards

the south the increase at the distant edge of the south field must have been independent of the apiary location. Various observers have noted a tendency for increased concentrations of bees near the outer edge of a field (Levin *et al.* 1960). The drop in the relative humidity around the plants, reflecting a change in the plant growth at this distance, indicates a possible increase in plant attractiveness contributing to the higher population density at this station.

Plants on the south field apparently became less attractive during the 3 days that data were collected, as evidenced by the day-to-day decrease in total bee population. Since the number of cordovans increased slightly while the total population was decreasing, the bees working there from the more distant apiary must have been leaving the field, although it was still somewhat attractive to the closer cordovans.

This explanation is supported by the work of Butler *et al.* (1943), who found a correlation between the ease with which bees were deterred from collecting sugar sirup by unfavorable conditions and the distance to the sirup dishes. Observations by Boch (1956) further emphasize the effect of crop attractiveness and distance on the foraging activities of bees.

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Short-Day Cotton Stocks as Possible Sources of Host Plant Resistance to the Pink Bollworm[1]

D. K. REED and PERRY L. ADKISSON,[2] *Texas Agricultural Experiment Station, College Station*

ABSTRACT

An improvement of a previously used technique for screening cotton stocks for pink bollworm (*Pectinophora gossypiella* (Saunders)) resistance was devised. The improved procedure involved the use of newly hatched pink bollworm larvae, rather than eggs, for infesting bolls of experimental stocks. Of the short-day stocks tested, *Gossypium hirsutum* race *palmeri* and *G. hirsutum* race *marie-galante* exhibited resistance to the pink bollworm. Further study indicated that the resistance of *marie-galante* was apparently due to the extensive proliferation of tissue in infested bolls which crushed, trapped or so injured the young larvae that death ensued. The resistance of *marie-galante* was categorized as antibiosis. The level of resistance occurring in F_1 hybrids of *marie-galante*×Deltapine 15 suggested that the genetic characters responsible for resistance exhibited partial dominance in the cross to the susceptible stock.

In the last decade there has been an intensive effort by several research workers to find possible sources of host plant resistance to the pink bollworm, *Pectinophora gossypiella* (Saunders). Brazzel (1956) screened many cotton stocks and found that *Gossypium thurberi* and the F_1 hybrid of a *G. hirsutum*×*G. tomentosum* cross appeared to possess some resistance to the insect. Pearson (1958) listed several stocks in which a degree of resistance has been reported. He mentioned reports of a high level of resistance in certain native cottons of Haiti and Puerto Rico which, from their description, must have been *G. hirsutum* race *marie-galante*.

Most of the previous work has been confined to day-neutral plants that bloom under the long summer days of the temperate zone. Because they will not bloom in the field during the normal temperate zone growing season, the "short-day cottons" have been mostly neglected in research for host plant resistance. Lewis & Richmond (1957) stated that since early breeding work in the United States was confined to material grown in the field during long days of summer, it is obvious that all nonflowering plants in introduced stocks were lost the very first year they were planted. Waddle *et al.* (1961) suggested that it was reasonable to expect that certain of these stocks may carry latent genes for valuable economic characters which might express themselves in the temperate zones.

This group of stocks represent a vast source of untested germplasm. It is possible that certain members of the group may possess resistance to some of the more important cotton insects since several of these stocks are prevalent in areas where certain of these pests have been established for many years.

PROCEDURE.—The stocks used in these experiments, together with identifying numbers and other pertinent information, are given below:

G. barbadense (PI 220040/CB 2933), a primitive cotton indigenous to the Andean valley of Bolivia and Colombia. This stock is short-day in fruiting habit. The common

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[2] Research Assistant and Associate Professor, respectively. Acknowledgement is made to Dr. S. P. Johnson, formerly Assistant Professor in the Department of Plant Physiology and Pathology for suggesting the inclusion of the short-day stocks in this research. The cooperation of Dr. Joseph Hacskaylo of the Department of Plant Physiology and Pathology and Dr. T. R. Richmond, Dr. G. A. Niles and Dr. Meta Brown of the Cotton Breeding Section, Texas Agricultural Experiment Station, is also gratefully acknowledged.

name for this cotton is Lengupa, by which it will be designated in this paper.

G. hirsutum race *latifolium* (Collectors No. MW 44, Texas No. 101), a day-neutral stock collected in Guatemala. Hereafter in this paper, this stock will be referred to as MW 44.

G. hirsutum race *palmeri* (Collectors No. MW 333, Texas No. 275), a short-day, nearly glabrous stock common to much of Mexico.

G. hirsutum race *marie-galante* (Collectors No. MW 372, Texas No. 373), a short-day cotton found in Guatemala, El Salvador, Colombia, Ecuador, the Caribbean Islands and Brazil.

G. hirsutum race *latifolium* variety Deltapine 15, a widely grown commercial variety that is susceptible to pink bollworm attack. This variety is day-neutral and was used as a standard for comparison.

The stocks were obtained from the Cotton Section, Department of Agronomy of the Texas Station. Preliminary tests were conducted in the greenhouse during the winter months with a random selection of short-day stocks. The plants were grown singly in pots. As blooms appeared they were dated and tagged in order that the ensuing bolls could be identified as to age group. When the bolls were from 10 to 20 days of age they were each infested with five viable pink bollworm eggs. Eggs were collected from standard oviposition cages and held for 4 days in an incubator at approximately 80° F. On the fifth day, they were separated for viability and transferred to the bolls by means of a soft camel's-hair brush. The eggs were held for the 5-day period since previous observations had indicated eggs of this age should hatch within a relatively few hours after being placed on the bolls. Approximately 7 days after infestation the bolls were bagged in plastic mesh bags. Approximately 20 days following infestation the bolls were removed from the plant and carefully examined for pink bollworm entrance holes, emergence holes and for living and dead larvae. Examinations were made with the aid of a binocular microscope. Per cent survival was computed by dividing the number of live insects recovered by the number of entrance holes.

Results of these greenhouse tests indicated that a more efficient method of infesting bolls was desirable. A technique using newly hatched larvae rather than viable eggs was evaluated. For this method eggs were held on filter paper in a glass vial at room temperature until they hatched. Five newly hatched larvae were transferred to each experimental boll by a soft camel's-hair brush. The larvae were confined to the bolls by means of a thin band of tanglefoot, which was applied to the pedicel of the boll.

Table 1.—Comparative resistance of various cotton stocks to the pink bollworm.

Cotton Stock	Number of						Survival (%)
	Bolls Infested	Entrance Holes	Emergence Holes	Larvae Recovered		Adults and Pupae Recovered	
				Living	Dead		
Lengupa	120	131	25	98	2	7	80.2
MW 44	79	31	1	25	3	0	80.6
Palmeri	13	6	0	0	3	0	0.0
Marie-Galante	49	23	1	3	19	0	13.0

Because the short-day cottons will not bloom naturally during long days special procedures had to be devised to induce fruiting during the summer. A light-proof shed, large enough to house three 6-×6-foot carts, was constructed. The carts were installed on tracks to facilitate moving. The selected cottons were planted in pots and placed on the carts. Each cart was covered with a plastic screen cage which served to exclude other insects. The cottons were exposed to the sunlight for 8 hours daily. This technique proved satisfactory as all the stocks under observation produced fruit.

RESULTS.—Preliminary studies indicated that MW 44 and Lengupa were susceptible and the *marie-galante* and *palmeri* stocks were resistant to the pink bollworm (table 1). Because of boll-size and certain other agronomic characters the *marie-galante* was selected for further testing.

Since the egg method of infestation employed in this test did not produce as high a level of infestation as the number of eggs used would indicate, studies were conducted to compare this procedure with one using newly emerged larvae. The data given in table 2 show that the larval technique was more efficient than the egg method. The larval method produced a great increase in the number of entrance holes per boll since a much higher percentage of the potential number of insects originally placed on the bolls attempted entry. This method was utilized for the summer tests.

In tests during the summer of 1959, MW 44, *marie-galante* and the F_1 hybrids of MW 44×Deltapine 15 and *marie-galante*×Deltapine 15 were tested against the Deltapine 15 standard. Results of these tests are presented in table 3. The data obtained from these experiments substantiated the conclusions drawn from the greenhouse tests, *i.e.*, the *marie-galante* stocks possessed considerable resistance to the pink bollworm. The 27% survival of pink bollworms on the F_1 hybrid of *marie-galante*×Deltapine 15 indicated a high level of resistance and suggests that the genetic characters involved exhibited partial dominance. The survival of pink bollworms

Table 2.—Comparison of egg-infestation and larval-infestation methods for screening purposes.

Cotton Stock	Egg Method				Larval Method			
	No. Infested Bolls[a]	No. Entrance Holes	Entrance Hole Per Boll	Insects to Attempt Entry (%)	No. Infested Bolls[a]	No. Entrance Holes	Entrance Hole Per Boll	Insects to Attempt Entry (%)
Marie-Galante	49	23	0.47	9.4	34	89	2.62	52.3
F_1	2	1	0.50	10.0	38	102	2.68	53.7
DPL 15	26	25	0.96	19.2	17	47	2.76	55.3

[a] Each boll was infested with either 5 viable eggs or 5 larvae according to the method employed.

Table 3.—Comparative resistance of various parental stocks and F_1 hybrids to the pink bollworm.

Cotton Stock	Number of						
	Infested Bolls	Entrance Holes	Emergence Holes	Larvae Recovered		Adults and Pupae	Survival (%)
				Living	Dead		
MW 44	30	31	6	26	0	4	96.8
MW 44×Deltapine	14	8	1	8	0	0	100.0
Marie-Galante	26	55	0	8	46	0	14.5
Marie-Galante×Deltapine	31	59	0	16	41	0	27.0
Deltapine	43	72	10	58	4	9	93.0

on the two *marie-galante* stocks was considerably below that recorded on MW 44 and Deltapine 15.

Careful examination of the infested *marie-galante* bolls revealed that several larvae had made entry into the carpel wall. Of the dead larvae recovered all were in the first or second instar. Of the eight live larvae recovered from this stock, only four had attained the last larval instar. This suggests a possible lengthening in the duration of the larval stage of the pink bollworms that were able to survive in these bolls.

It was also found that the tissue of the boll carpels of the *marie-galante* stocks proliferated extensively around the injury made by the invading larvae. Most of the dead larvae were recovered either within the mass of proliferated tissue or between the proliferated tissue and the seed cotton lock. This would suggest that the proliferation of tissue was responsible for larval mortality simply by crushing them, trapping them or injuring them to the extent that death ensued. A similar reaction of boll tissue to an invading insect was reported by Hinds (1906) in work with *Anthonomus grandis* Boheman.

Slides of carpel tissue surrounding the larval entrance holes in the *marie-galante* bolls were examined microscopically. Within 48 hours of infestation, there was partial to complete autolysis of the walls of the cells surrounding the injury. This was accompanied by cellular hypertrophy and the development of masses of amorphic granular-appearing tissue in the localized area surrounding the entrance hole. The proliferated tissue was exuded through the larval entrance hole and masses of such tissue were formed on the external and internal surfaces of the boll carpel. Preparations made from carpel tissue of the susceptible Deltapine 15 variety indicated no such phenomenon.

In some cases, however, dead larvae of the early instars were recovered well within the boll capsule of the *marie-galante* stocks and beyond the proliferated tissue. This may indicate that factors other than proliferation may also be involved. Regardless, the resistance exhibited by the *marie-galante* stocks can be categorized as antibiosis as defined by Painter (1951).

Discussion.—The data presented suggest that the short-day cottons could prove to be valuable sources of germplasm for insect resistance. Of the four stocks studied, two, *palmeri* and *marie-galante*, appeared to exhibit some resistance to the pink bollworm. Work by Lewis & Richmond (1957) and Waddle *et al.* (1961) has shown the short-day response of these stocks does not, in itself, eliminate these cottons from breeding programs in the temperate zone. This response can be eliminated in segregating generations when crosses are made with day-neutral stocks. The short-day stocks probably represent one of the largest sources of untested germplasm in the United States. The results presented indicate they should receive much more attention in future studies concerned with host plant resistance. Preliminary screening of a number of these stocks is presently being conducted at this laboratory.

The resistance exhibited by the *marie-galante* stocks apparently was due largely to the extensive proliferation of the carpel tissue surrounding the larval entrance holes. If this characteristic is enough in itself to produce some degree of resistance it is possible that the genetic characters responsible for such a reaction are present in other stocks, some of which may prove to be less difficult than *marie-galante* to manage in breeding programs. The results obtained with the *marie-galante* are sufficient to warrant further research with this stock. Preliminary crosses of *marie-galante* to a commercial upland variety have been made. The resulting hybrids and backcrosses of the hybrids to each parent are now undergoing tests at this laboratory.

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Lindane Residues in Chickens and Eggs Following Poultry House Sprays[1]

M. C. Ivey, R. H. Roberts, H. D. Mann, and H. V. Claborn, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

Abstract

Studies were made to determine lindane residues in the fat and eggs of chickens confined in houses sprayed with 1% suspensions of the insecticide. Two experiments were conducted at Kerrville, Texas. In the first, during the summer of 1958, the interior of a poultry house was thoroughly sprayed at the rate of 1 gallon per 100 square feet. In the second, during the winter of 1959–1960, a light application was made at the rate of 1 gallon per 1000 square feet. The heavy application caused residues in the fat that averaged 131 p.p.m. at 1 week after the house was sprayed and 97 p.p.m. at 16 weeks. The residues in the eggs varied from 13 to 20 p.p.m. during 12 weeks, but no decrease was evident at 12 weeks. Although the light application caused lower amounts of lindane in both fat and eggs, significant residues were still present in the eggs 16 weeks and in the fat 20 weeks after the house was sprayed.

The recommendation of the Entomology Research Division (1959) for the use of lindane to control ectoparasites of poultry necessitated a residue study to determine how much of this insecticide was stored in the fat of chickens and in eggs and how long any such contamination remained. The recommendation given was to spray house, roosts, and nests thoroughly with 0.5% to 1.0% lindane emulsion or suspension.

Two residue experiments were conducted at Kerrville, Texas, the first in the summer of 1958, and the second during the winter of 1959–1960. In the first experiment the interior of a chicken house was heavily sprayed, whereas in the second the house was lightly treated.

Methods and Materials.—For both studies a 1% lindane suspension formulated from a wettable powder (25% concentration) was used to spray walls, ceiling, roost, and nests. In 1958 the insecticide was applied at the rate of approximately 1 gallon per 100 square feet. In 1959–1960 the rate of application did not exceed 1 gallon per 1000 square feet. At the time of treatment the chickens, feeders, and water and shell pans were removed. Before treatment, six chickens were sacrificed for fat samples and 1 dozen eggs pooled for controls. After treatment, samples of fat and eggs were taken at intervals for residue analyses.

The heavy application was made in a poultry house that was 10 feet 2 inches square and had a sloping roof 8 feet 3 inches high in front and 6 feet 3 inches at the rear. The front of the building was covered with net wire. The wooden floor had a layer of wood shavings 4 inches deep. This litter was not sprayed directly but was contaminated by spray drift and dripping.

Four chickens were sacrificed weekly for the first 3 weeks after treatment, and two chickens at 4, 6, 8, 12, and 16 weeks. Egg samples were taken weekly. All the eggs gathered on a sampling day were pooled, mixed, and frozen; a weighed sample was taken for analysis.

Twelve weeks after treatment six untreated hens were obtained from another source. Two were sacrificed immediately for control fat samples, and four were placed in the poultry house. At the same time two chickens were moved from the poultry house to an untreated coop. Sixteen weeks after treatment two of the chickens that had been in the poultry house only 4 weeks and the two that had been moved to the untreated coop were sacrificed.

The second treatment was applied to a poultry house 30 by 100 feet. The building was well ventilated; one long side was screened with chicken wire to a height of 7 feet. No litter was used on the dirt floor. Four chickens were sacrificed and six eggs taken weekly for 6 weeks after treatment and then at 8, 10, 12, 16, and 20 weeks for residue analyses.

Analytical Procedures.—The colorimetric method of Schechter & Hornstein (1952) was used for the analysis of the fat and egg samples. To get low and constant blanks and satisfactory recoveries it was necessary to modify the method as described below.

Analysis of Chicken Fat.—A 10-gram sample of fat was extracted by blending with anhydrous sodium sulfate and chloroform. The filtered chloroform extract was concentrated to 200 ml. and gently shaken with 8 ml. of a 1:1 mixture of sulfuric acid-fuming sulfuric acid. When the sulfonated fatty layer had separated, the chloroform was drained off and the fatty layer washed twice with chloroform. The combined chloroform extracts were extracted twice with 35 ml. of the fuming sulfuric acid mixture. The chloroform was transferred to a clean separatory funnel, washed once with 5% sodium bicarbonate and twice with distilled water, dried over sodium sulfate, and concentrated to 10 ml. by distillation through a Snyder column. The residue was transferred to a 125-ml. Erlenmeyer flask with 50 ml. of acetic acid. A Snyder column was inserted in the flask and a thermometer was suspended in the Snyder column so that the bulb was just below the top. The solvent was evaporated until the temperature reached the boiling point of acetic acid and then was cooled to room temperature. The column was removed and 3 grams of malonic acid and 4 grams of 40-mesh zinc were added to the flask. The flask was attached to the Schechter-Hornstein still and the described method followed.

Analysis of Eggs.—A 25-gram sample of the frozen eggs was weighed out into a beaker and mixed with 75 ml. of water and 15 ml. of alcohol. The mixture was transferred to a 500-ml. separatory funnel and extracted twice with 200 ml. and once with 100 ml. of a 75:25 ethyl ether-acetone mixture. The combined extracts were washed three times with water and dried over sodium sulfate. The solvent was removed by distillation through a Snyder column. The residue was dissolved in 200 ml. of chloroform, extracted once with 50 ml. of concentrated sulfuric acid and three times with 50 ml. of a 1:1 mixture of sulfuric acid and fuming sulfuric acid. The chloroform was transferred to a clean separatory funnel and washed with 5% sodium bicarbonate and distilled water. From this point the method described for the analysis of the fat was followed.

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Table 1.—Parts per million of lindane in the fat and eggs from chickens confined in a poultry house following a heavy spray treatment with 1.0% lindane suspension.[a]

	Weeks After Spraying							
Sample	1	2	3	4	6	8	12	16
Fat	72	128	87	68	159	175	129	74
	168	168	87	198	121	275	182	120
	176	109	110					
	110	66	83					
Average	131	118	92	133	140	225	156	97
Eggs	13	13	16	16	13	20	15	—

[a] One gallon per 100 square feet.

chickens were 4 p.p.m. after 4 weeks. The two chickens placed in the treated house 12 weeks after spraying showed 54.2 and 46.9 p.p.m., respectively, of lindane after 4 weeks of confinement.

The residues found in the fat and eggs from chickens confined in the house given the light spray treatment are shown in table 2.

The results of these two experiments show that significant residues of lindane are present in chickens and eggs following treatment of a poultry house with lindane. The residues persisted for more than 16 weeks following the heavy spray and for more than 20 weeks following the light spray.

On the basis of the results obtained in these experiments, the recommendation for the use of lindane to control poultry ectoparasites has been withdrawn.

Table 2.—Parts per million of lindane in the fat and eggs from chickens confined in a poultry house following a light spray treatment with 1.0% lindane suspension.[a]

	Weeks After Spraying										
Sample	1	2	3	4	5	6	8	10	12	16	20
Fat	4.9	3.3	2.5	2.2	3.7	1.2	4.0	1.4	0.3	0.7	0.7
	4.3	7.8	7.8	4.3	2.2	2.1	3.6	1.1	1.0	0.4	1.4
	3.5	8.6	5.7	1.4	6.0	1.2	10.4	1.9	0.5	0.5	0.9
Average	4.2	6.6	5.3	2.6	4.0	1.5	6.0	1.4	0.9	0.5	1.0
Eggs	0.53	0.81	0.51	0.57	—	0.31	0.33	0.16	0.16	0.08	—

[a] One gallon per 1000 square feet.

The above procedures gave recoveries of 87% to 98% when known amounts of lindane were added to the control fat and 80% to 91% when lindane was added to the control eggs.

RESULTS.—The residues of lindane found in the fat and eggs from the chickens confined for 16 weeks in the poultry house treated with the heavy spray treatment are shown in table 1.

The residues present in two chickens 4 weeks after removal from the poultry house were 27.7 and 58.8 p.p.m. respectively. The residues present in the eggs from these

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Influence of Formulation on the Effectiveness of Malathion, Methoxychlor, and Synergized Pyrethrum Protective Sprays for Stored Wheat[1]

R. G. Strong, D. E. Sbur, and R. G. Arndt,[2] *University of California Citrus Experiment Station, Riverside*

Abstract

Laboratory tests were made in a study to determine the degree of toxicity and repellency to the rice weevil, *Sitophilus oryzae* (L.), and the confused flour beetle, *Tribolium confusum* Duv., of selected dosages of malathion (5 p.p.m.), methoxychlor (15 p.p.m.), and synergized pyrethrum (pyrethrins—1.5 p.p.m. +piperonyl butoxide—15 p.p.m.) applied to wheat in sprays formulated as solutions, emulsions, and wettable-powder suspensions; the residues remaining on wheat at various intervals after application of sprays; the proportional amount of residue on the wheat related to each formulation that is removed by the usual handling and cleaning practices followed at milling; the residues finally resulting in the milled fractions; and the effect on quality of bread as evaluated by taste and odor panels. Two temperature levels and two levels of moisture content were incorporated in the series of tests composed of 36 primary lots of treated wheat and an equivalent amount of unsprayed wheat for appropriate controls.

Differences between formulations in the effectiveness of insecticides applied in sprays as protectants against insect infestations in stored wheat were not clearly defined, but differences between the two species of test insects in their response to protective treatments were of particular significance. *S. oryzae* appeared to be more susceptible to the toxic effects of insecticides, and *T. confusum* appeared to be extremely sensitive to repellent action.

In tests with malathion, *S. oryzae* was more susceptible than *T. confusum* as indicated by results from toxicity tests. The influence of moisture content and storage temperature on the persistence of biologically effective malathion deposits on wheat was recognized by reduced mortalities of test insects with increases in temperature and moisture content. Repellency indicated for malathion applied in the tetrachloroethylene solution formulation was considered to be due primarily to the solvent used. Residues of malathion persisting on whole grain at various intervals after application of spray decreased with time; the influence of moisture content and storage temperature on the persistence of residues was indicated by the results from chemical analyses for residues on whole grain. In milled samples the larger amounts of malathion residue were recovered from bran (including germ), shorts, and wheat middlings. Very little malathion carried over into the flour.

Methoxychlor treatments failed to kill insects in toxicity tests and demonstrated little or no repellency toward the rice weevil, but they were highly effective in repelling confused flour beetles. Larger amounts of methoxychlor residues were recovered from bran than from other milling fractions, but considerable methoxychlor was recovered from flour.

Negative results were obtained in toxicity tests with *T. confusum* exposed to wheat sprayed with the synergized pyrethrum formulations. Erratic results, but recognizable toxic effects, were recorded in tests with *S. oryzae*. Mortalities of *S. oryzae* declined rapidly in tests made after storage of synergized-pyrethrum-treated wheat for more than 1 month after application of sprays. Results from repellency tests conclusively illustrated repellency of the synergized pyrethrum sprays to both species of test insects. Residues persisting on whole grain at various intervals after application of spray decreased with time. Results from chemical analyses for piperonyl butoxide indicated that cleaning operations used in this study were not highly efficient in removing chemical residues from treated wheat, probably because most of the insecticide retained by wheat was in the pericarp and endosperm. Residues recovered were greater in bran than from the other milled fractions, but recoveries from flour were considerably higher than expected.

Small differences observed between treatments in flavor and odor evaluation of bread made with flour milled from wheat treated with the insecticides tested were not considered of significance.

Extraneous materials such as lime, wood ashes, sulfur, silica gel, diatomaceous earth, and salt have been used with the intention of preventing insect infestations in stored grain. Reasons are obvious for objection to such matter being put on grain intended for human consumption and animal feeds, although their value as protectants against insect infestations may not be denied without ample exploration in carefully designed experiments. More recently protective dusts and sprays have been used that incorporate small amounts of an insecticide which kills or repels insects but is relatively harmless to man and domestic vertebrates in the quantities applied. Diluents for insecticides in dust formulations must be selected with discretion in order to avoid objectionable deposits of inorganic matter. The use of sprays might advantageously reduce the necessity for concern about residues to merely those of insecticides remaining after application.

Although the use of protective sprays on wheat appears to be a promising method of preventing insect infestations during long periods of storage, it is expedient to use minimum effective deposits of an insecticide in a formulation that will be removed to the greatest degree by the milling process in order to keep insecticidal residues in milled products at the lowest possible level. Lack of information on the effectiveness of a given dosage of insecticide applied in various spray formulations, the degree to which the insecticide in such formulations penetrates into the wheat kernel, and the proportional amount

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of residue removed by normal handling and cleaning processes at milling led to a study of the performance of selected insecticides applied to wheat as protective sprays formulated as solutions, emulsions, and wettable-powder suspensions.

Selection of insecticides for use in this study was based on previously reported indications of their effectiveness as grain protectants. Reports suggesting that pyrethrum synergized with piperonyl butoxide might be used for protection of stored grain against insect infestations were made by Watts & Berlin (1950), Wilbur (1952 a, b), Eden (1953), and Schroeder (1955). Treatments were not effective in all tests, however, and Apt (1954) failed to inhibit development of dangerous insect populations with sprays and dusts containing a mixture of piperonyl butoxide and pyrethrum. LaHue *et al.* (1958) suggested the need for higher dosages than had been used previously. Walkden & Nelson (1959) and Chapman (1960) found synergized pyrethrum effective for long periods. The initial report of Lindgren *et al.* (1954) on the use of malathion as a grain protectant was followed by several papers (Parkin 1958, LaHue *et al.* 1958, Schesser *et al.* 1958, Gunther *et al.* 1958, Womack & LaHue 1959, Watters 1959, Strong & Sbur 1960, Chapman 1960) containing pertinent information on dosages, influence of moisture and temperature, chemical residues, and persistence of biological effectiveness for the protection of grain against damage by storage pests. Methoxychlor dusts protected stored wheat and shelled corn from insect infestations over long periods of storage in Kansas and Missouri; dosages as low as 5 p.p.m. on wheat gave protection for one full summer season (Walkden & Nelson 1958). Dosages of more than 100 p.p.m. were required for protection of shelled corn with methoxychlor dusts in the Southeast (LaHue *et al.* 1958, Womack & LaHue 1959), and emulsions were not effective. Variations between reports on minimum effective deposits of insecticide handicapped the selection of a specific dosage of each insecticide to spray on wheat for assessment of the influence of formulations.

It is the purpose of this paper to present a summary of results obtained from laboratory tests made in a study to determine the degree of toxicity and repellency to the rice weevil, *Sitophilus oryzae* (L.), and the confused flour beetle, *Tribolium confusum* Duv., of selected dosages of three insecticides applied to wheat in sprays formulated as solutions, emulsions, and wettable-powder suspensions; the residues remaining on wheat at various intervals after application of sprays; the proportional amount of residue on the wheat related to each formulation that is removed by the usual handling and cleaning practices followed at milling; the residues finally resulting in the milled fractions; and the effect on quality of bread as evaluated by taste and odor panels. Two temperature levels and two levels of moisture content were incorporated in the series of tests composed of 36 primary lots of treated wheat and an equivalent amount of unsprayed wheat for appropriate controls.

MATERIALS AND METHODS.—White Federation, a variety of western white wheat, was purchased locally. Careful adjustment of moisture and conditioning of wheat was required to obtain samples with the desired moisture content of 10% and 13%. The moisture content was determined with an "*EH*" *Universal Moisture Tester* made by the Moore-Milford Corporation, Evanston, Illinois. Initial moisture tests indicated the need for addition of water or drying. When addition of water was necessary, moistened wheat was thoroughly blended in a concrete mixer. To reduce the moisture content, wheat was dried in narrow wire-screen containers placed in a forced-air oven maintained at 125° to 130° F. Following each moisture adjustment, wheat was conditioned in sealed containers (similar to E in fig. 1) for 2 weeks. Then moisture tests were made. Addition of water or drying, alternated with the conditioning period before moisture tests, was continued until the desired percentages of moisture were obtained.

Conditioned wheat was divided into lots for treatment according to insecticide, formulation, moisture content, and storage temperature. Each formulation of each insecticide was sprayed on four lots of wheat, considered as "primary lots" in this study. One lot of 10% moisture content was stored at 60° F. and another at 90° F. One lot of 13% moisture content was stored at 60° F. and another at 90° F. Samples were withdrawn at intervals of 1 day, 2 weeks, 1, 3, 6, 9, 12, 15, 18, 21 and 24 months following application of spray, or until a low degree of toxicity and repellency was reached. Treatments for primary lots of wheat were not replicated.

A simple apparatus (fig. 1) was constructed to deliver grain at a constant rate during application of sprays. Wheat left the hopper (A) through gates (B) at the bottom and fell onto an agitator (C) which moved it rapidly into a trough (D). Spray was applied into the grain stream as wheat flowed from the trough into a storage container (E). An ordinary compressed air sprayer (fig. 1, F) with a capacity of 1.5 gallons was modified for use in spraying grain. By controlling air pressure on the line with a regulator (not shown) and equipping the tank with a gauge to indicate pressure, spray could be applied to wheat at a constant and exact rate.

Wettable-powder suspensions were applied at the rate of 236 ml. (260 ml. of synergized pyrethrum wettable-powder suspension), emulsions at the rate of 236 ml., and tetrachloroethylene solutions at the rate of 208 ml. per 100 pounds of wheat. Although 10 times the amount of spray actually needed was prepared, insecticides were used according to amounts needed in the spray when applied to a known amount of wheat to give the desired deposits of 5 p.p.m. malathion, 15 p.p.m. methoxychlor, and 1.5 p.p.m. pyrethrins (synergized with piperonyl butoxide applied at the rate of 15 p.p.m.). Formulations of spray containing water caused a slight increase in the moisture content of treated wheat.

Malathion, methoxychlor, and piperonyl butoxide-pyrethrum mixture were purchased as technical insecticide, wettable powders, and emulsifiable concentrates from local suppliers. Malathion spray formulations were prepared with 25% malathion wettable powder, emulsifiable concentrate containing 5 pounds of technical malathion per gallon, and technical malathion in tetrachloroethylene. Methoxychlor wettable-powder suspension was prepared with 50% methoxychlor wettable powder, emulsion with an emulsifiable concentrate containing 2 pounds of technical methoxychlor per gallon, and the solution formulation was made with emulsifiable concentrate dissolved in tetrachloroethylene. Pyrenone® Wettable Pow-

FIG. 1.—Application of protective sprays to wheat with calibrated equipment.

der 20-2 containing 20% technical piperonyl butoxide and 2% pyrethrins was used to prepare wettable-powder suspensions of synergized pyrethrum. The emulsion formulation was made with an emulsifiable concentrate prepared from Pyrenone O.F. 75–7.5 (75% piperonyl butoxide–7.5% pyrethrins) by using 80 gm. of this concentrate and 20 gm. of Atlas G 1045-A emulsifier. This gave an emulsifiable concentrate containing 60% technical piperonyl butoxide and 6% pyrethrins. Pyrenone O.F. 75–7.5 was dissolved in tetrachloroethylene to prepare the solution formulation.

Each primary treatment lot of wheat was contained in two 100-pound cans which were sealed with masking tape after application of spray. An equivalent amount of untreated wheat was placed in storage in the same manner. Storage containers were opened only for sampling or the removal of grain for milling. Temperatures were thermostatically controlled at 60° ± 5° F. and 90° ± 5° F. in large walk-in constant-temperature chambers serving as storage rooms. Sprayed and unsprayed wheat was stored separately but at the required temperatures.

All wheat was sampled for toxicity tests, repellency tests, and chemical residue analysis in the same manner. To avoid contamination in the checks, unsprayed grain was always sampled first. The two cans of treated grain for each lot were removed from storage and placed side by side. Grain was first removed from the center of the test containers, then from four points at the side of each container. Probes were alternated from one container to another at each sampling point. As wheat was removed it was placed on waxed paper until a sufficient amount had been taken. The complete sample was placed in clean glass containers. All samples were blended for 15 minutes in a stainless-steel twin-shell blender of 8-quart capacity. Blended subsamples of 500 grams each were submitted for chemical residue analysis. Subsamples were also taken

after blending for use in toxicity and repellency tests.

The toxic effectiveness of the protective treatments was tested at intervals designated for aging of deposits by confining 20 adults each of *S. oryzae* and *T. confusum* in approximately 20 gms. of wheat contained in cloth-covered wide-mouth 2-oz. glass jars. Five replications were made for each test with each species of insect used. Paired comparisons were made by using an equivalent amount of unsprayed wheat. After an exposure of 14 days during which the test samples were kept in a testing room with a temperature of 80° ± 1° F. and a relative humidity of 60% ±5%, the wheat was screened and insect mortalities recorded.

The method used for repellency tests at each of the designated test intervals was described by Laudani & Swank (1954). Containers with treated and untreated grain were alternated in the apparatus. Two chambers were used for each lot of wheat tested at each interval, giving a total of 12 treated and 12 untreated samples for each repellency test. One hundred and fifty individuals of the rice weevil and the same number of confused flour beetles were introduced into each testing apparatus. These were left for 18 hours before recording the number of insects found in treated and untreated samples.

Rice weevils were reared in wheat with an initial moisture content of 14.5%. Culture media for the confused flour beetle was ground to pass through a 20-mesh screen and consisted of a mixture by weight of whole wheat flour—30 parts, corn meal—10 parts, wheat germ—1 part, dried skim milk—1 part, and brewers yeast—½ part. Parent insects were left in cultures for an oviposition period of 7 days. Insects were used for toxicity and repellency tests 14 days after the first adults appeared in cultures, giving test insects with a mean adult age of 7 days and a range in age of 1 to 14 days. The temperature in the culture room was maintained at 80° ± 2° F. and the relative humidity at 60% ± 5%.

Analyses for chemical residues on whole grain were made at intervals after treatment of 1 day, 1, 3, 6, 12, 18, and 24 months, or until the grain was removed from tests. The procedure used initially for chemical determination of malathion residues on grain was that of Norris *et al.* (1954) as described by Gunther & Blinn (1955). However, owing to difficulties experienced in achieving consistent recoveries from fortified samples, modification of the procedure described by Conroy (1957) was substituted for use in the latter stages of residue analysis for malathion. Methoxychlor residues were determined by a colorimetric procedure adapted from Schiffman's report (1957). Piperonyl butoxide determinations were based on the procedure proposed by Jones *et al.* (1952), and pyrethrins were assumed to be present in the original ratio of pyrethrins to piperonyl butoxide applied to wheat.

Wheat for milling was treated separately. Two 50-pound samples were used for each test lot, one sprayed and one unsprayed. Three months after application of insecticide, wheat was withdrawn from storage for milling. One day prior to milling in a Buhler mill available for test use in the laboratory of a private milling company, wheat was tempered by increasing the moisture content to 14% as required in the milling process.

Two 1500-gm. samples, one check and one treated, were withdrawn from their respective storage containers for each lot of wheat and milled to obtain white flour. One loaf of bread was made with flour from each sample. The bread was evaluated for flavor and odor by a panel of judges.

Samples taken at the mill for chemical residue analysis included wheat before cleaning, dockage as removed by an Emerson dockage tester and Forester scourer, clean wheat (after cleaning and before milling), and milled products from the Buhler mill as follows: 1st, 2nd, and 3rd break flour; 1st, 2nd and 3rd reduction or middling flour; bran, including germ, shorts, and low grade or wheat middlings.

Milled wheat fractions were processed by the usual methods in the laboratory to afford final stripping solutions. Analyses for chemical residues were made on the following samples: dockage, bran, shorts, low grade or middlings, and a composite of the flour samples (aliquots taken from 1st, 2nd, and 3rd break flour, and 1st, 2nd, and 3rd reduction flour).

RESULTS.—The biological effectiveness and residual persistence of selected dosages of each of three insecticides applied to wheat in sprays formulated as emulsions, wettable-powder suspensions, and tetrachloroethylene solutions were determined by toxicity and repellency tests and chemical analysis for residues remaining on grain at designated intervals after application of sprays. Residue analyses made on samples collected during milling operations indicated the proportional amount of residue on wheat related to each formulation that was removed by the usual handling and cleaning practices followed at milling and the insecticide finally resulting in milled fractions. In general, only a summary of results essential to the understanding and interpretation of results is presented in this report. However, those data not presented graphically or in tabular form are mentioned when necessary for clarification.

Results from toxicity tests were corrected by Abbott's formula (1925) and the corrected percentage mortalities of test insects are presented graphically or in tables. Data obtained from repellency tests are summarized in deviation histograms which indicate the variation in percentages of insects found infesting treated and untreated wheat under the conditions of the tests. From the total number of insects of each species found in wheat after examination of repellency-test samples, the percentages occurring in treated and untreated control samples were calculated. Differences in percentages of insects infesting sprayed and unsprayed wheat are shown as the deviation of treated from untreated checks. Positive or negative symbols indicate larger or smaller percentages of insects found in treated than in the corresponding untreated control samples of wheat used in repellency tests. Data presented for results obtained from chemical residue analyses are those submitted by technicians performing the analyses after corrections were made, when necessary, for background and recovery. Evaluations made on flavor and odor of bread by panels of judges are mentioned briefly.

Malathion Formulations.—Results from tests concerned with the influence of formulation on the toxic and repellent effectiveness of malathion and persistence of chemi-

cal residues on whole grain are given in figure 2. Graphs depicting results from toxicity tests with *S. oryzae* and *T. confusum* project the corrected percentages of mortality (numbers on top of bars) caused by 5 p.p.m. malathion on wheat of 10% and 13% moisture content after storage at 60° and 90° F. for the periods shown (age of deposit) after application of sprays formulated as emulsion, wettable-powder suspension, and tetrachloroethylene solution. Results obtained from repellency tests are indicated in deviation histograms. Differences between percentages of insects in treated and untreated control samples are shown as deviations from checks. The tabulation of results from chemical residue analyses indicates the amount of malathion (p.p.m.) persisting on grain at various intervals after application of sprays. Data in table 1 indicate the proportional amount of malathion residue on wheat related to each spray formulation that was removed by handling and cleaning practices followed at milling and the insecticide finally resulting in milled fractions.

The data presented in graphical and tabular form in figure 2 permit comparisons of toxicity and repellency of malathion spray formulations with the related residue found by chemical analysis from the time of treatment through completion of tests. Further summary comparisons between other variables, such as moisture content and storage temperature, can be made at a glance. Comparison of results given in table 1 with those in figure 2 suggests the possibility of correlating toxicity of malathion to certain stored-product insects with residues which may be expected in wheat milling fractions.

Small differences noted in flavor tests were not significant and indicated that malathion treatments did not result in a detectable flavor effect. The slight differences among treatments and untreated checks with respect to flavor probably would be of no practical significance. There was, however, evidence that the more sensitive panel members detected an odor in certain treated samples. This was apparently due to causes other than malathion treatments as all bread samples derived from wheat sprayed with malathion, or any one formulation of malathion spray, did not have a detectable odor.

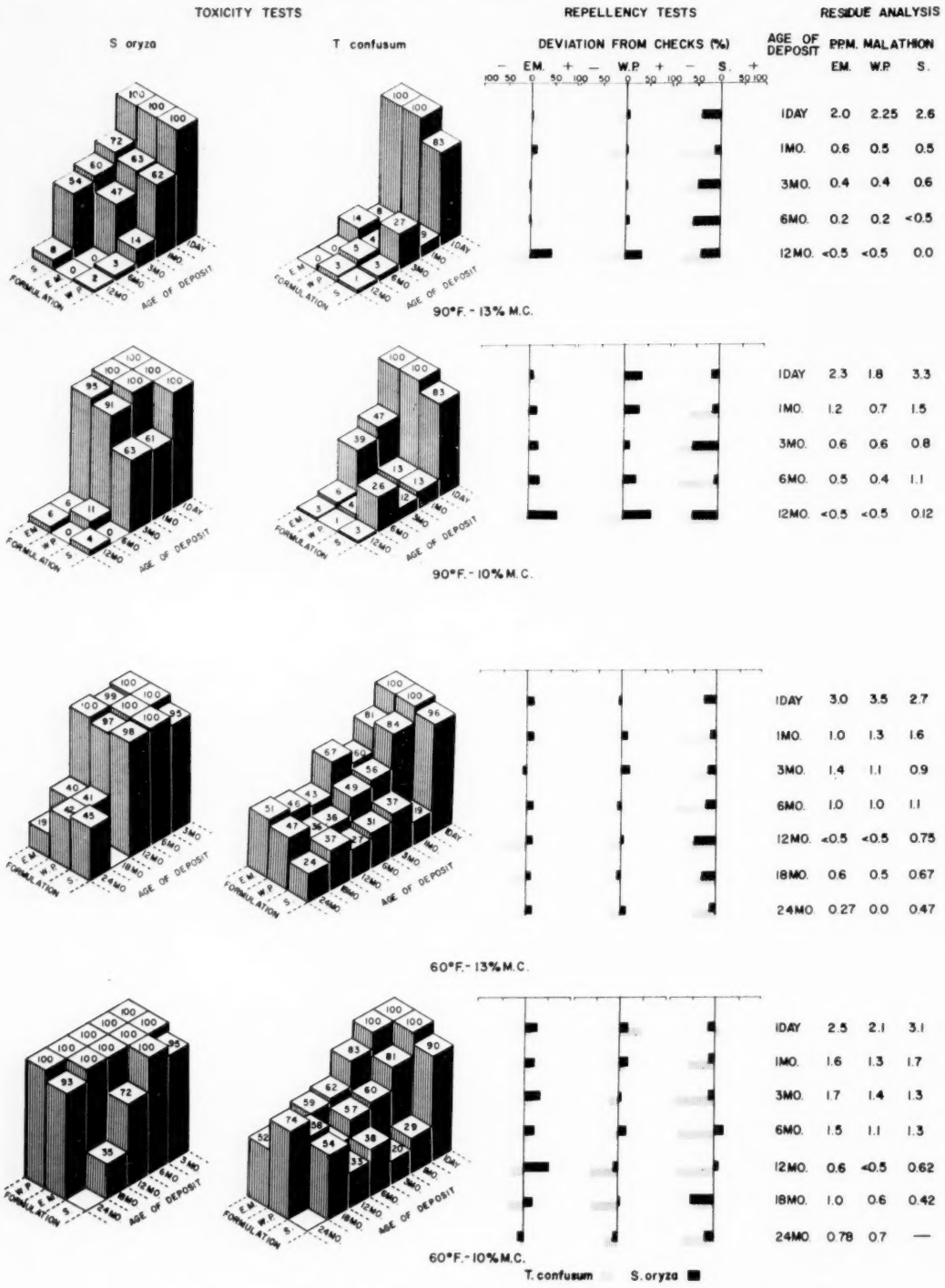
Methoxychlor Formulations.—Mortality of test insects exposed to methoxychlor-treated wheat was negligible as indicated by the summary of results in table 2 from toxicity tests made 1 day after application of sprays. Similar results were obtained from other tests made after aging of deposits for various periods. Results from tests concerned with the influence of formulation on the repellent effectiveness of methoxychlor and persistence of chemical residues on whole grain are given in figure 3. Differences between percentages of insects in treated and untreated control samples in repellency tests are shown graphically as deviations from checks. The tabulation of results from chemical residue analyses indicates the amount of methoxychlor (p.p.m.) persisting on grain at various intervals after application of sprays. Data in table 1 indicate the proportional amount of methoxychlor residue on wheat related to each spray formulation that was removed by handling and cleaning practices followed at milling and the insecticide finally resulting in milled fractions.

Slight differences among the treatments and untreated checks found in flavor tests with bread would probably be of no practical significance. There was no indication that the methoxychlor treatments resulted in odor differences. There was, however, an indication that in comparison with other samples two loaves of bread were inferior. This was not considered to be related to the methoxychlor treatments.

Synergized Pyrethrum Formulations.—Results from tests concerned with the influence of formulation on the toxic and repellent effectiveness of pyrethrins synergized with piperonyl butoxide and the persistence of insecticidal residues on whole grain are given in figure 4. The results from toxicity tests are summarized as the per cent mortality of test insects exposed to treated grain at various intervals (age of deposit) after application of sprays. Differences between percentages of insects found in treated and untreated control samples of wheat used in repellency tests are indicated in the graphic summary of results as deviations from checks. The tabulation of results from chemical residue analysis indicates the amount of piperonyl butoxide (p.p.m.) persisting on grain at various intervals after application of sprays. Data in table 1 indicate the proportional amount of piperonyl butoxide residue on wheat related to each spray formulation that was removed by handling and cleaning practices followed at milling and the insecticide finally resulting in milled fractions. Chemical analyses for pyrethrins were not made, but, for the purpose of this study, it was assumed that synergized pyrethrum maintained its original ratio (1 to 10) of pyrethrins to piperonyl butoxide. On this basis results from chemical analyses for piperonyl butoxide can be used to estimate residues of pyrethrins on wheat. Small differences noted in flavor and odor tests with bread were not significant and indicated that the treatments did not result in a detectable flavor effect.

DISCUSSION.—Parkin (1956), in his review of stored-product entomology, severely criticized the tendency for the practical use of contact insecticides for the control of stored-product insects to outstrip properly contrived experimental work, resulting in difficulty at times in drawing the line between scientific contributions and pseudoscientific papers that are little more than advertisements. One suspects that Parkin was not chagrined by the favorable outcome of result demonstrations but was trying to warn of difficulties ahead with continued use of insecticides for the control of stored-product insects without ample exploration of ways in which most effective results may be obtained with minimum residues on grain and in the finished cereal products.

In the statement of the purpose of this paper it is apparent that the present study attempted to cover broad areas in which information was lacking. The success of this attempt to rapidly bridge the gap between laboratory tests, in the strictest sense, and practical experiments or demonstrations in the field may be measured largely by the results and discussion presented. Although time and facilities available restricted certain details, the results obtained are in keeping with the purposes of the study. Indications or trends observed in the data presented in this report should give direction for additional work, delineate areas needing more urgent attention, and, in some cases, offer an explanation for differences between results reported previously by other investigators.



FORMULATIONS: EM. = EMULSION, W.P. = WETTABLE POWDER, S. = SOLUTION (TETRACHLOROETHYLENE)

Table 1.—Residue of insecticides found during milling and in the milling fractions of wheat 3 months after applying initial deposits of malathion (5 p.p.m.), methoxychlor (15 p.p.m.), and synergized pyrethrum (1.5 p.p.m. pyrethrins and 15 p.p.m. piperonyl butoxide) in various spray formulations.

Formulation	Storage Temperature (° F.)	Moisture Content of Wheat (%)	Recovery of Insecticide (p.p.m.) from Whole Grain: Before Cleaning	Whole Grain: After Cleaning	Whole Grain: Clean-out (dockage)	Milling Fractions from Buhler Mill: Flour (composite)	Bran	Shorts	Low Grade (middlings)
				Malathion					
Emulsion	90	13	0.4	—	3.6	<0.5	2.4	0.9	0.5
		10	0.6	—	6.1	<0.5	3.9	<0.5	<0.5
	60	13	1.4	—	10.9	0.2	5.0	0.9	0.6
		10	1.7	—	10.9	<0.5	5.1	0.7	0.6
Wettable-powder suspension	90	13	0.4	—	1.8	0.1	1.6	0.7	0.2
		10	0.6	—	2.8	<0.5	2.4	<0.5	<0.5
	60	13	1.1	—	6.1	0.11	4.1	0.2	0.5
		10	1.4	—	9.1	<0.5	5.8	0.8	0.7
Tetrachloroethylene solution	90	13	0.87	0.65	1.79	0.00	7.69	1.67	1.90
		10	0.66	0.50	1.41	0.00	12.28	4.72	1.83
	60	13	2.07	0.85	1.76	0.27	11.40	2.99	3.51
		10	1.07	1.35	2.12	<0.67	10.30	2.93	2.04
				Methoxychlor					
Emulsion	90	13	11.6	—	83.8	4.4	25.4	3.4	<2.0
		10	4.2	—	82.0	4.0	23.6	2.8	<2.0
	60	13	11.9	—	94.4	6.6	28.4	11.6	3.6
		10	11.7	—	82.0	4.0	26.6	4.4	<2.0
Wettable-powder suspension	90	13	10.8	—	101.6	5.4	23.4	5.8	—
		10	8.8	—	79.2	6.4	27.1	3.6	<2.0
	60	13	12.0	—	101.2	5.8	18.0	<2.0	<2.0
		10	12.1	—	89.0	6.6	16.4	<2.0	<2.0
Tetrachloroethylene solution	90	13	7.1	6.6	37.09	1.65	14.59	9.81	2.58
		10	5.41	6.12	19.76	2.59	20.96	13.38	5.06
	60	13	6.8	4.0	73.30	4.77	13.18	9.06	5.65
		10	7.3	5.41	37.38	2.47	23.22	8.66	4.61
				Piperonyl Butoxide[a]					
Emulsion	90	13	6.04	4.84	38.80	1.65	12.17	0.149	2.53
		10	6.21	5.53	29.34	3.30	13.00	7.20	9.10
	60	13	6.08	3.90	18.76	0.11	10.99	2.31	4.18
		10	7.3	8.63	20.53	1.92	10.88	4.78	2.64
Wettable-powder suspension	90	13	11.64	3.58	31.20	<0.45	10.62	<0.45	2.25
		10	5.4	—	29.20	3.20	14.61	7.5	1.00
	60	13	9.04	7.61	26.93	2.90	12.17	0.11	5.34
		10	10.91	8.75	25.26	4.26	7.56	8.36	4.21
Tetrachloroethylene solution	90	13	4.07	4.35	5.65	3.20	9.51	4.30	3.26
		10	6.74	4.51	13.70	4.57	13.76	5.00	—
	60	13	7.87	8.59	34.66	2.40	7.17	1.41	1.63
		10	5.43	4.19	8.32	4.53	11.43	1.90	1.12

[a] For the purpose of this study it was assumed that synergized pyrethrum maintained its original ratio of pyrethrins to piperonyl butoxide (1 to 10) and that recoveries of piperonyl butoxide by chemical analysis could be used to estimate residues of pyrethrins.

FIG. 2.—Influence of spray formulation on the toxic and repellent effectiveness and persistence of residues of malathion applied at 5 p.p.m. to White Federation wheat of 10% and 13% moisture content after storage at 60° and 90° F. for various periods (age of deposit) as indicated by corrected percentage mortalities of test insects (numbers on top of bars in graphs depicting results from toxicity tests), deviations in the percentage of insects found in treated from those in untreated control samples of wheat used in repellency tests, and the related amounts of malathion (p.p.m.) recovered by chemical residue analysis of whole grain samples.

Table 2.—Percentage mortality of test insects after exposure for 14 days to wheat treated with 15 p.p.m. methoxychlor 1 day before assay.

Spray Formulation	Storage Temperature (° F.)	Moisture Content of Wheat (%)	*S. oryzae*	*T. confusum*
Emulsion	90	13	3	0
		10	0	0
	60	13	6	4
		10	0	0
Wettable-powder suspension	90	13	0	1
		10	8	1
	60	13	0	0
		10	0	4
Tetrachloroethylene solution	90	13	28	1
		10	7	1
	60	13	1	0
		10	0	0

Before proceeding with the discussion of results from tests with various formulations of specific insecticides, techniques used for toxicity tests, repellency tests, and analysis for chemical residues are mentioned in order to avoid misinterpretation of the data presented. Procedures described for toxicity tests are similar to those employed by many investigators, and results obtained may be duplicated without serious difficulty. The methods used for chemical analyses for insecticidal residues were those considered to give the greatest accuracy in the presence of grain and cereal products. Lack of replication of primary treatments, however, was a serious handicap to the analysts in this phase of the work. Results from repellency tests are more apt to be misunderstood.

The method described by Laudani & Swank (1954) did not give a highly sensitive test of repellency. Gross effects were recognized easily from results obtained; the weakness of the method seemed to lie in its failure to accurately measure the degree of repellency so comparisons could be made accordingly. In preliminary trials it was observed that duplication of results from one day to another would be difficult, if not impossible. Nevertheless, the Laudani & Swank method has merit in being the only recognized laboratory-test-method presently available for assessment of repellency to stored-product insects of protective treatments on grain

The results obtained from repellency tests have been summarized in simple deviation histograms to indicate repellent effects of treatments at various intervals after application of sprays and to facilitate comparisons between formulations. No deviation between treated and untreated controls when equal numbers of insects or percentages of insects were found is indicated by the absence of a bar in charts depicting results from repellency tests. When more insects were found in treated than in untreated control samples, the difference in percentages of insects is shown by a bar to the right or positive (+) side of the median or zero line of the chart. Such results indicate a lack of repellency, possibly due to the toxic action of the insecticide applied. When fewer insects were found in treated than in untreated control samples, the difference in percentages of insects is indicated by a bar to the left or negative (−) side of the median or zero line and indicates repellency of the treatment. Small deviations are not considered significant.

Malathion Formulations.—It was noted in previous reports from this laboratory (Strong & Sbur 1960, 1961) that *T. confusum* was less susceptible to malathion than *S. oryzae*. Pronounced differences between the two species in susceptibility to malathion treatments are seen in the results presented in figure 2 from toxicity tests at various intervals after application of sprays. Results from tests with *S. oryzae* are more distinct, and, in general, changes are more abrupt than with *T. confusum*.

Superiority of a particular formulation is not evident from the results of toxicity tests summarized in figure 2. From the viewpoint of protection against *S. oryzae* and *T. confusum* by toxic action, failure of any one formulation of malathion to give consistently superior results voids distinction between formulations on the basis of the data presented. One example should suffice as an explanation. The solution formulation appeared to be the most effective against *S. oryzae* in wheat of 13% moisture content but the least effective in wheat of 10% moisture content.

The influence of moisture content and storage temperature on the persistence of biologically effective malathion deposits on wheat was recognized by reduced mortalities of test insects with increases in temperature and moisture content. Differences in the effectiveness of malathion in protecting wheat against insects under varying conditions of moisture content and storage temperature, so readily apparent in the summary of results from toxicity tests presented in figure 2, were indicated in the early stages of this study. Trends observed stimulated a more comprehensive investigation by Strong & Sbur (1960) on the influence of moisture content and storage temperature on the effectiveness of malathion as a grain protectant.

Results from repellency tests (fig. 2) suggest that insects were repelled by malathion in tetrachloroethylene solution. Repellency indicated for this formulation, however, probably should be attributed more to the solvent used than the insecticide. The practice of sealing containers prevented escape of the solvent during storage for aging of deposits. The presence of tetrachloroethylene was obvious during each sampling and test period. Perhaps of more significance in the results from repellency tests is the indication of a tendency for *T. confusum* to be repelled by malathion in all formulations in tests made at the end of 12, 18, and 24 months after application of sprays. This point is worthy of further investigation. Persistence of biological effectiveness of a toxic substance in preventing infestations of stored-product insects after dissipation of its lethal action would be of considerable importance. Transition from toxic to repellent action of a grain protectant might be recognized by reduction in mortalities of test insects with a direct increase in repellency. Or the change may be more subtle, requiring considerable ingenuity in devising techniques for valid assessment of results.

Residues of malathion (fig. 2) persisting on whole grain at various intervals (age of deposit) after application of spray decreased with time. The influence of moisture and storage temperature on the persistence of malathion

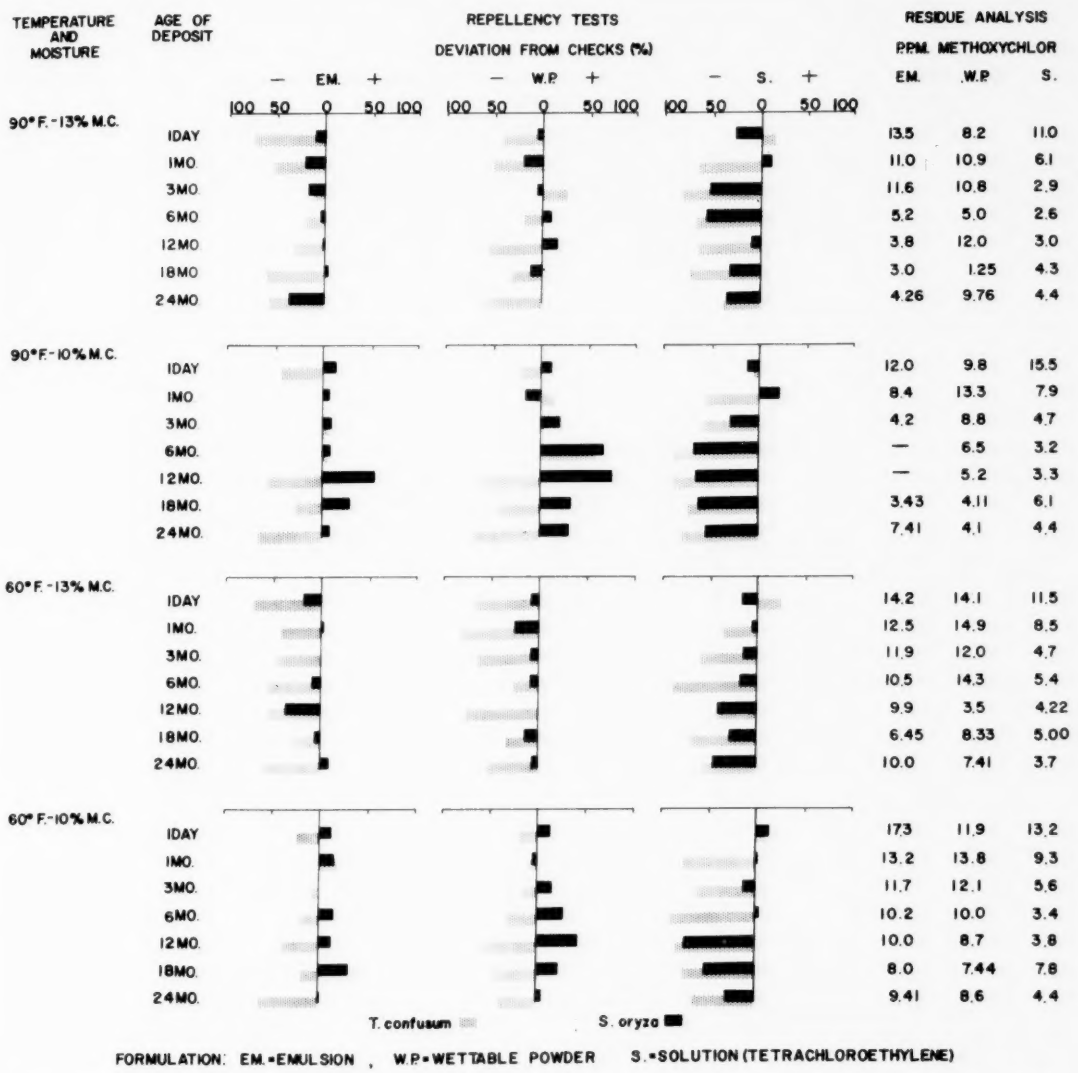


FIG. 3.—Influence of spray formulation on the repellent effectiveness and persistence of residues of methoxychlor applied at 15 p.p.m. to White Federation wheat of 10% and 13% moisture content after storage at 60° and 90° F. for various periods (age of deposit) as indicated by deviations in the percentage of insects found in treated from those in untreated control samples of wheat used in repellency tests and the related residue of methoxychlor recovered by chemical analysis.

residues is indicated by the results from residue analyses. Data presented in table 1 summarizing results from chemical analyses for malathion residues during milling and in the milled fractions of wheat agree in general with results from similar work reported by Schesser *et al.* (1958). The data indicate that cleaning removed only a small amount of malathion, residues of insecticide found in cleanout or dockage is less an indication of cleaning efficiency than of the relative amounts of dockage in each lot of wheat, and, in milled samples, the larger amounts of chemical residue were in the bran (including germ), shorts, and wheat middlings. Very little malathion carried over into the flour. Residues recovered from the bran of wheat sprayed with malathion in tetrachloroethylene solution were considerably higher than from the other formulations.

Methoxychlor Formulations.—From results of toxicity tests summarized in table 2 it is obvious that methoxychlor sprayed on wheat at the rate of 15 p.p.m. failed to control either species tested under the conditions of this study. These data provide ample reason for a more thorough review and analysis of the results and discus-

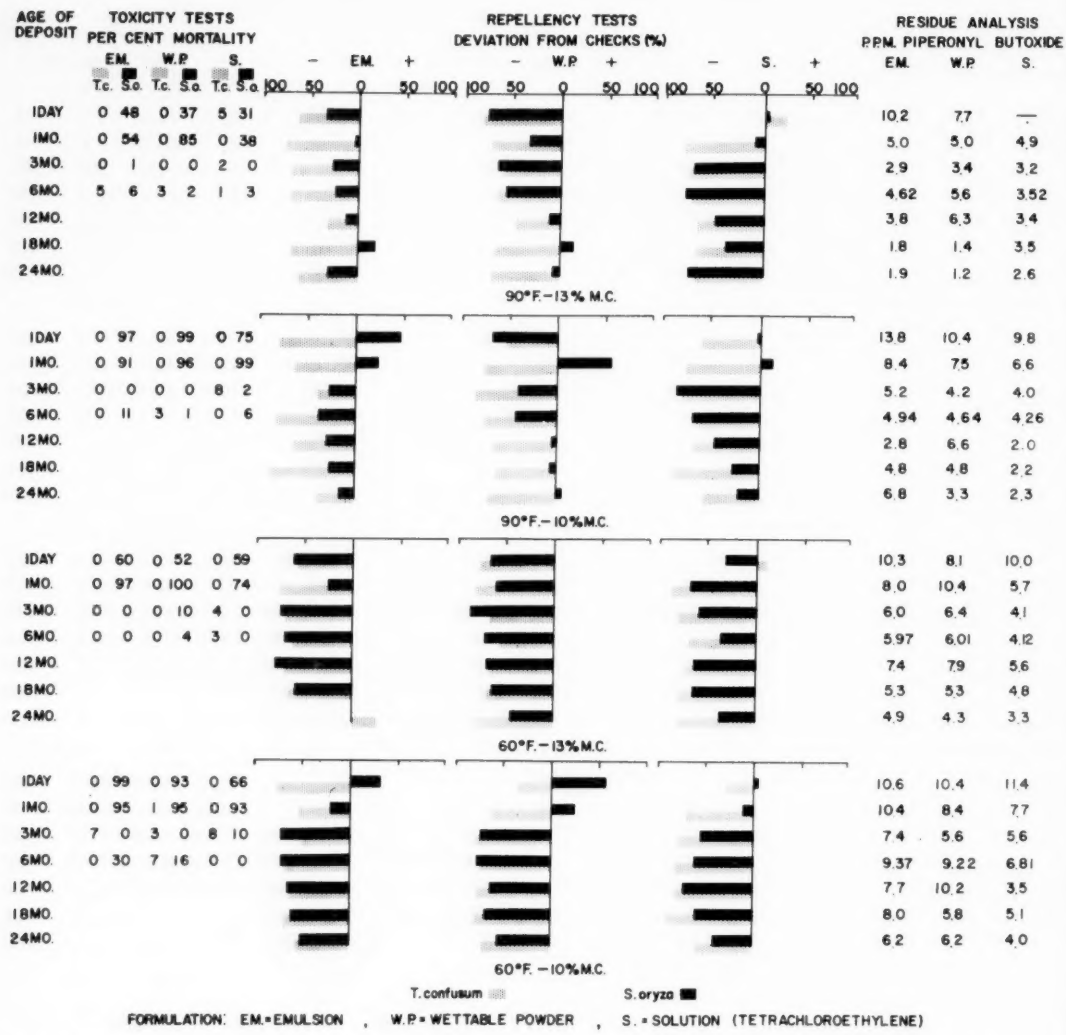


FIG. 4.—Influence of spray formulation on the toxic and repellent effectiveness and persistence of residues of pyrethrins (1.5 p.p.m.) synergized with piperonyl butoxide (15 p.p.m.) applied to White Federation wheat of 10% and 13% moisture content after storage for various periods (age of deposit) as indicated by corrected percentage mortalities of insects in toxicity tests, deviations in the percentage of insects found in treated from those in untreated control samples of wheat used in repellency tests, and the related amounts of insecticide residue recovered by chemical analysis for piperonyl butoxide (assuming that pyrethrins remained in the original ratio of 1 to 10).

sions presented in the three papers dealing with methoxychlor as a grain protectant which were cited earlier in this report.

In tests with methoxychlor dusts applied at the rate of 5, 10, and 15 p.p.m. of insecticide on wheat in large bins, Walkden & Nelson (1958) noted that at all three levels of methoxychlor treatments the insect populations were practically eliminated following application of protectants. The bins remained almost free of insects the following season. Although higher dosages of methoxychlor in dust formulations were used for treatment of shelled corn, a statistical analysis of results indicated that dosage levels of 54 p.p.m. methoxychlor and higher were greater than needed for effective protection against insect infestations over the 30-month observation period.

LaHue *et al.* (1958) used varying dosages of methoxychlor on corn in exploratory tests in the Southeast. Their results indicated that application rates of 100 to 200 p.p.m. would be necessary for effective protection of shelled or husked corn with methoxychlor dusts. In later work by Womack & LaHue (1959) it was demonstrated that effective protection of shelled corn in the Southeast

with methoxychlor would require dosage rates of 100 p.p.m.

Bioassay tests reported by Walkden & Nelson (1958) supporting data from their practical large-bin studies resulted in high percentages of mortality of *S. oryzae* adults exposed to methoxychlor-treated corn. Owing to rather high mortalities of *S. oryzae* in untreated control samples, however, the results reported from treated corn may not be considered as conclusive evidence of the toxic effectiveness of methoxychlor treatments.

The variation between results in the reports mentioned in the preceding discussion indicated a need for strict evaluation of methoxychlor and other insecticides under controlled laboratory conditions in order to establish a logical basis for selection of minimum dosages of insecticides to be applied in more detailed laboratory or practical investigations. In a preliminary study made for this purpose by Strong & Sbur (1961), it was found that 100 p.p.m. methoxychlor in acetone solution was required to obtain 100% mortality of adult *S. oryzae* exposed to treated wheat 1 day after application of spray. In wheat sprayed with 200 p.p.m. methoxychlor, the mortality of other species of test insects was reported as follows: *Sitophilus granarius* (L.) adults—81%, *Tribolium confusum* adults—4%, and larvae of the khapra beetle, *Trogoderma granarium* Everts —5%. Confronted with these data it is apparent that reasons other than toxicity of methoxychlor to stored-product insects are required to explain differences observed between results obtained in practical studies with methoxychlor as a grain protectant.

Results from repellency tests with methoxychlor as summarized in figure 3 of this report may give the logical explanation needed to bridge the gap between somewhat negative data from toxicity tests in the laboratory and conclusive protective action of low dosages of methoxychlor observed in practical tests. Reasons for differences in dosages reported as being required for protection of corn in the Southeast as compared with the Middle West may be projected after careful analysis of the results in figure 3 and further consideration of reports concerned with the use of methoxychlor as a grain protectant.

In the discussion of techniques used in this study, it was mentioned that gross effects were easily recognized from results obtained with the repellent-test-method employed; quantitative measurement of the degree of repellency obtained could not be made. For this reason results from repellency tests summarized graphically in figure 3 can only be assessed qualitatively. At least part of the repellency indicated for methoxychlor in tetrachloroethylene solution should be attributed to the solvent rather than the insecticide for the same reasons given for similar results obtained with this formulation of malathion spray. An examination of results for the emulsion formulation and wettable-powder suspension indicates little or no repellency of methoxychlor to *S. oryzae*. Presumably, if it had been methoxychlor that repelled this species in the solution formulation, a higher degree of repellency should be indicated in the results obtained with other formulations. It was concluded from the results presented that methoxychlor was not highly effective as a repellent against *S. oryzae* under the conditions of the tests. In most tests for repellency the response of *T. confusum* adults to methoxychlor-treated wheat differed radically from *S. oryzae*, and the effectiveness demonstrated by methoxychlor treatments in repelling *T. confusum* is illustrated in the summary of results given in figure 3.

An influence of temperature on the repellent effectiveness of methoxychlor is not shown in the results from repellency tests in figure 3, but moisture effects are suggested by the data presented from tests with *S. oryzae*. Higher percentages of *S. oryzae* were found in treated samples of wheat of 10% moisture content, at both 60° and 90° F., than in wheat of 13% moisture content. The results indicate increased toxicity of methoxychlor at the lower moisture level, but results from toxicity tests already had shown this not to be the case. Another explanation is that on wheat of lower moisture content more residues of methoxychlor remained than on wheat of higher moisture content, and, because of paralysis or other toxic effects short of lethal action, *S. oryzae* entering treated samples of wheat were unable to escape. Unfortunately, data from residue analyses for methoxychlor on whole grain are too irregular to be of value in correlating results for more valid conclusions.

Differences observed in the response of two species of insects to methoxychlor in repellency tests suggest that differences between results obtained by Walkden & Nelson (1958) in comparison with those reported by LaHue *et al.* (1958) and Womack & LaHue (1959) might be explained after an examination of their respective reports for a listing of the species of insects they were concerned with in their studies. Although the comparative abundance of the species of insects infesting corn was not specifically mentioned by LaHue *et al.* or by Womack & LaHue, an examination of tables in these two reports is sufficient to indicate that *S. oryzae* was the most prevalent species of stored-product insects encountered in their investigations.

Walkden & Nelson (1958) presented a rather revealing discussion on the comparative abundance of the species of insects in their studies. Five species were found in wheat; flat grain beetles (probably a complex of three species: *Laemophloeus pusillus* (Schönh.), *L. ferrugineus* (Steph.), and *L. turcicus* Grouv.) were by far the most abundant, followed by the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), and the lesser grain borer, *Rhyzopertha dominica* (F.). These were the predominant species present in tests in which methoxychlor treatments applied at the rate of 5, 10, and 15 p.p.m. were highly effective. Although 17 species of stored-grain insects were found in corn during the 30-month test period, the *S. oryzae* was the most abundant in bins of shelled corn treated with methoxychlor. This species comprised 45% of the total insects observed as compared with little more than 1% of the total insects in untreated control bins. The authors stated that reasons for this situation were not clear. The most abundant species in untreated corn was the red flour beetle, *Tribolium castaneum* (Hbst.), followed by flat grain beetles and the saw-toothed grain beetle. Nearly 90% of the total insects observed in the control bins of corn were of these species.

After consideration of these reports one might logically suggest that Walkden & Nelson found low dosages of methoxychlor effective in protecting grain against storage insects because of the repellent action of the insecticide. The predominance of *S. oryzae* in corn treated with

methoxychlor compared with the minute infestations found in untreated controls suggests that the use of methoxychlor and its subsequent failure to kill or repel *S. oryzae*, combined with a high degree of repellency to other stored-product insects, upset the normal balance of populations of insects found in bins of corn. Perhaps there was even a proportionate increase in the population of *S. oryzae* which could have been correlated with the repellent action of methoxychlor against other important species of insects found infesting corn, especially those species dominating populations in untreated control bins. LaHue *et al.* and Womack & LaHue were primarily concerned with the control of *S. oryzae* by toxic action for the protection of corn in the Southeast. For this reason, higher dosages of methoxychlor were required.

Although larger amounts of methoxychlor residue were recovered from bran (including germ) than from other milling fractions (table 1), residues found in flour were considerably higher than those reported by Schesser *et al.* (1958) from results obtained in similar studies. The differences observed may have been due, at least in part, to the differences between facilities used in the cleaning operations and milling procedure.

Synergized Pyrethrum Formulations.—Differences between *Tribolium confusum* and *S. oryzae* in susceptibility to the toxic effects of synergized pyrethrum sprays are observed in the summary of results from toxicity tests in figure 4. Under the conditions of this study, sprays containing pyrethrins (1.5 p.p.m.) synergized with piperonyl butoxide (15 p.p.m.) were not effective against *T. confusum;* erratic results, but recognizable toxic effects, were recorded in tests with *S. oryzae*. Similar results were obtained when these tests were repeated. Mortalities of *S. oryzae* declined rapidly in tests made after storage of treated wheat more than 1 month after application of sprays.

Results from repellency tests summarized graphically in figure 4 give conclusive evidence that *S. oryzae* and *T. confusum* are strongly repelled by pyrethrins synergized with piperonyl butoxide. All formulations were effective in repelling both species of insects. Minor inconsistencies probably should be attributed to a lack of refinement in the test method. Differences observed between *T. confusum* and *S. oryzae* in their response to treated wheat should be considered in more detail. Results recorded from repellency tests made 1 day and 1 month after application of spray, especially with *S. oryzae*, were more erratic than those from tests made after longer periods of storage. This suggests that the toxic effect of the treatment interfered.

An influence of moisture content on repellency of the synergized pyrethrum sprays is not suggested by the data presented. However, the influence of temperature on the effectiveness of treatments is indicated by the results from tests with *S. oryzae*. With the exception of the tetrachloroethylene solution in which the solvent may have contributed to repellent action under the conditions of this study, results from tests with *S. oryzae* on treated wheat stored at 90° F. as compared with that stored at 60° F. appear to be significantly different.

Residues of piperonyl butoxide (fig. 4) persisting on whole grain at various intervals (age of deposit) after application of spray decreased with time. The influence of moisture and storage temperature on the persistence of piperonyl butoxide residues is indicated, but the results from residue analyses are too variable for definite conclusions. Data presented in table 1 summarize results from chemical analyses for piperonyl butoxide residues during milling and in the milled fractions of wheat. The data indicate that cleaning operations used in this study were not highly efficient in removing chemical residues from treated wheat, probably because most of the insecticide retained by wheat after cleaning was in the pericarp and endosperm. In milled samples, residues of piperonyl butoxide recovered were greater in bran (including germ) than from the other fractions. Residues found in flour were considerably higher than expected.

For the purpose of this study it was assumed that synergized pyrethrum maintained its original ratio of pyrethrins to piperonyl butoxide (1 to 10) and that recoveries of piperonyl butoxide by chemical analysis could be used to estimate residues of pyrethrins. Blinn *et al.* (1959), in a study of the residual behavior of pyrethrins and of piperonyl butoxide on stored wheat, found pyrethrins to have an apparent residue half-life of 5.8 weeks in contrast with a half-life of 9.9 weeks for piperonyl butoxide under the conditions of their investigation. The authors concluded that the practice of analyzing for piperonyl butoxide residues on the assumption that pyrethrins content maintains its original ratio with piperonyl butoxide is not valid, but this practice can be condoned for purposes of defining toxicological hazard if the actual residual relationship of these two materials is considered.

Comments.—Differences between formulations in the effectiveness of malathion, methoxychlor, and synergized pyrethrum sprays as protectants on stored wheat were not clearly defined in toxicity and repellency tests with *S. oryzae* and *T. confusum*, or in the results of chemical residue analyses. These results were in sharp contrast with those reported by Kantack & Laudani (1957) in which wettable-powder suspensions were strikingly superior to emulsion formulations on treated surfaces when exposed to Indian-meal moth (*Plodia interpunctella* (Hbn.)) larvae and adults. The substrate involved was probably of considerable significance in determining the outcome of both studies. Comparison of results from these studies is sufficient to indicate the necessity for clearly defining the purpose of each study in which protectants are used against stored-product insects in order to avoid confusion in interpretation of results. Otherwise, the results from the studies mentioned above may have appeared to be conflicting rather than supplementary.

Differences observed in the results obtained in this study between the two species of test insects in their reponse to protective treatments are of particular significance. *S. oryzae* appeared to be more susceptible to the toxic effects of insecticides, and *T. confusum* appeared to be extremely sensitive to repellent action. Indeed, repellency demonstrated by methoxychlor treatments against *T. confusum* was surprising, and the results obtained in these tests were enlightening. Obviously, more consideration should be given to parallel toxicity and repellency tests in the laboratory for the evaluation of insecticides as grain protectants. Perhaps of equal importance is the necessity for testing insecticides being considered for use

as grain protectants against a wide range of species of stored-product insects.

In view of the discussion in this paper on differences between results reported from tests with methoxychlor by various workers, more attention should be given to the designation of species, varieties, or forms of test insects. This might prevent confusion later. For example, Floyd & Newsom (1959) found the large and the small rice weevils to be distinct, reproductively isolated species. The name *Sitophilus oryzae* (L.) was considered to be applicable to the former and *S. sasakii* (Tak.) to the latter. The small or lesser rice weevils were used in this study.

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Egg Parasitism and Larval Habits of the Orange-Striped Oakworm[1]

STEPHEN W. HITCHCOCK, *The Connecticut Agricultural Experiment Station, New Haven*

ABSTRACT

Parasitism by *Trichogramma* and *Tetrastichus* of orange-striped oakworm (*Anisota senatoria* (J. E. Smith)) eggs varied from an average of 22.9% to 74.4% in various areas of Connecticut. The effect of these parasites was greater, however, than a mere count of their numbers showed. Gregarious early instar orange-striped oakworm larvae are less able to survive if their numbers are reduced. Laboratory tests over a 5-week period showed that 72.7% of the total mortality of these oakworms reared singly occurred in the first week as contrasted with 5% of the total mortality occurring in the first week when 16 larvae were reared together.

Some oakworm egg masses in the field were nearly 100% parasitized. When parasitism reduced the number of viable eggs, the first-instar larvae were less able to survive because reduced numbers affected their ability to move and feed.

In 1957 there were scattered infestations of the orange-striped oakworm (*Anisota senatoria* (J. E. Smith)) in Connecticut. The following year there was an unprecedented outbreak which resulted in the defoliation of about 37,000 acres (Hitchcock 1958). In the course of this outbreak, studies were initiated to gain some understanding of the biology and control of this pest.

The egg stage of this insect is of relatively short duration, ranging from 7 to 10 days (Felt 1905) or up to 12 days in Connecticut. Despite this short period of time, the egg stage may be heavily attacked by natural enemies which help to reduce the population. There are few reports of insects preying on the eggs of the orange-striped oakworm. Beal (1952) reported a species of *Anastatus* and Girault (1911) reported *Trichogramma minutum* Riley parasitizing the eggs. Felt (1905) observed *Podisus placidus* Uhl. feeding on the eggs.

METHODS.—Surveys were made at regular intervals throughout the infested area to observe the population of the pest and its associated natural enemies. Egg counts were made by bringing leaves bearing egg masses into the laboratory to be counted and examined for parasitism.

Larvae were reared by placing them in wide-mouthed 1-quart jars and providing fresh washed oak leaves for food. In survival tests at various population levels, the caterpillars were reared with 1, 2, 4, 8, and 16 larvae per jar from the first larval instar to pupation. Several replicates were run at each level. If a larva died it was replaced with another of the same instar.

RESULTS AND DISCUSSION.—Although there have been a variety of statements in the literature concerning the color of the eggs of the orange-striped oakworm (Becker 1938, Lugger 1890, Beal 1952), in Connecticut they are yellow when first deposited then lighten in color and develop a red ring. In the later stages the young larvae may be seen within the egg. In Connecticut, the females almost always oviposited on trees of the black oak group rather than the white oak group. The larvae, however, fed freely and were able to develop on any kind of oak. Larval development took place slowly and uncertainly on American chestnut. Other types of foliage tested were not suitable.

Table 1.—Parasitism by *Trichogramma* and *Tetrastichus*[a] in eggs of the orange-striped oakworm.

LOCALITY AND YEAR	AVERAGE NO. EGGS PER MASS	PARASITIZED EGGS (%)
South Windsor 1959	127.2	58.9
Moosup 1959	216.0	35.6
Sterling 1959	94.0	22.9
Norwich 1959	97.4	34.5
Voluntown 1959	63.5	74.4
South Windsor 1960	100.4	23.9
Moosup 1960	191.8	29.4

[a] Recovered from Voluntown only.

The number of eggs deposited by the females varied from place to place within the infested area (table 1). The area of newest infestation at Moosup had the greatest average number of eggs per egg mass whereas the moths of a declining population at Voluntown produced the fewest. Lack of proper food for the developing larvae of the parent generation in an area of heavy defoliation may partially account for this fact (Miller 1957). Although the number of eggs deposited on a leaf varied greatly, the largest number observed anywhere in a single egg mass was 403 at Norwich and even there the average egg mass size was only 97.4. This is considerably less than the 350 to 675 eggs per cluster observed many years ago in Minnesota (Lugger 1890).

The oakworm eggs were heavily attacked by *Trichogramma pretiosa* Riley (det. J. C. Schread) in all areas (table 1). At Voluntown the eggs were also parasitized by *Tetrastichus* sp. (det. P. B. Dowden) which increased the percentage of parasitized eggs to 74.4%. As the rate of parasitism was so much higher when both parasites were present, it is probable that the effect of the *Tetrastichus* was additive rather than replacing *Trichogramma*. Otherwise the *Trichogramma* parasitism ranged from 23% to 59%.

Table 1 shows a precipitous decline in the percentage of parasitized eggs in South Windsor between 1959 and 1960. It may be significant that a spray was applied to this area in 1959 to control orange-striped oakworm larvae. The spray was poorly timed and did not produce a good reduction of the oakworm population. It is possible, however, that it reduced the numbers of egg parasites so that they had not recovered by the following year. By contrast, the egg parasitism at Moosup (table 1) remained relatively static during this same period although no direct comparison between these two areas is possible. The rate of parasitism in South Windsor in 1959 was by far the highest of any area where only one parasite was present and some of the decrease in 1960 may have reflected a natural return to more normal levels of parasitism. There did not appear to be any direct relationships between parasitism and size of egg mass or number of masses present.

A further loss in the egg population was due to preda-

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Table 2.—Per cent of total mortality of larvae of the orange-striped oakworm occurring in any one week.

No. of Larvae per Jar	Week				
	1	2	3	4	5
1	72.7	18.2	0	9.1	0
2	75.0	18.8	0	0	6.2
4	47.1	20.6	8.8	5.9	17.6
8	0	23.3	16.7	10.0	50.0
16	5.0	21.7	5.0	28.3	40.0

tion. At Moosup, *Podisus* sp. caused an 8.2% loss which could be added to the 29.4% loss from parasitism (table 1), but in other areas egg predation apparently played only an insignificant role. The pattern of predation was not evenly borne by all egg masses but fell very heavily on a few. Once the predator found an egg cluster it tended to remain there and feed until the eggs were decimated.

Infertile eggs represented only a small proportion of the total number of eggs deposited. The percentage of infertile eggs ranged from 0% to 1.2% in various egg masses and averaged 0.38% overall. It thus did not represent an appreciable error when counting numbers of eggs for predictive purposes during infestations.

It was observed in the course of field work that larval development took place more slowly in some areas than others. At Voluntown in an area of very heavy egg parasitism and which had had outbreaks the past few years, there were many caterpillars which did not manage to survive the first stadium. It was considered that the gregarious habit of the young larvae might confer some advantage on them which the older more solitary larvae did not need.

The results of larval laboratory rearing experiments are summarized in table 2, showing the percentage of the total number of insects that died each week, *i.e.*, of all the insects that died over a period of 5 weeks at a population level of one, 72.7% died the first week. The results indicate that a larger percentage of the mortality of larvae in small populations took place in the early stadia whereas very little loss occurred in the early stadia at the higher population levels. At population levels through four larvae per colony, the greatest part of the mortality took place during the first 2 weeks; whereas at levels of eight or more larvae per colony, almost no deaths occurred in the first week. It might be expected that the critical population level at which heavy losses took place would be slightly higher under natural conditions. Unfortunately the declining population did not provide a large enough number of egg masses the following year to test this hypothesis adequately, but preliminary investigations indicated that it was probably slightly under 10 larvae per colony.

In the laboratory, many of the very young larvae in the small populations died when they fell from the oak leaves and were unable to crawl back up onto the leaves. Almost none of the very young larvae in the larger populations died in this manner. In observing the young larvae move over a smooth surface, it was noticed that a fine film of silk was spun. Likewise, if a larva crawled to the end of a stick resting against glass, it would pause at the end of the stick and move its head back and forth in a searching movement leaving a fine film of silk on the glass surface. The same fine film spun on a leaf surface by all the larvae enabled any one larva to maintain its footing. On a smooth glass surface, no larva would venture beyond this silk film and the movement of the colony was dependent on the abilities of all of its members to provide silk. Older larvae did not need this aid. A single larva also appeared to have difficulty biting into the leaf margin whereas with a group of larvae, once the leaf margin had been broken, all fed freely.

Thus at Voluntown, probably the greatest single source of error in population counts was the mechanical loss of very young larvae from the oak leaves. As discussed above, the mortality rate in small populations of very young larvae may be quite large because of the inability of the insects to maintain themselves in position and feed on the leaf. In the actual egg counts at Voluntown there were several egg masses in which the parasitism approached or reached 100%. It is extremely doubtful if the remaining individuals hatching from these eggs could survive, even in the absence of natural enemies. The parasitism of eggs by *Trichogramma*, which was augmented by *Tetrastichus*, could thus result, when it reached a high percentage, in a loss of young larvae which would not occur at the lower rates of parasitism, causing a greater reduction in population than might be assumed from a mere count of the parasitized eggs.

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Dasiops alveofrons, A New Pest of Apricots in California[1]

H. R. Moffitt[2] and F. R. Yaruss[3]

Abstract

Observations on the seasonal history and habits of *Dasiops alveofrons* McAlpine (Diptera: Lonchaeidae), a new pest of apricots in California, are presented along with a brief discussion of other lonchaeid flies of economic importance as primary insect pests. The fly attacks immature and mature fruits in a manner similar to that of the true fruit flies (Trypetidae). Characters serving in the identification of the various life stages are listed.

Of the many species comprising the dipterous family Lonchaeidae few are recorded as occurring as primary crop pests, with the great majority occurring as secondary invaders of damaged or decaying vegetable matter (Bezzi 1918, Blanchard 1948). Of those species occurring as primary pests *Lonchaea chalybea* Wied. (Cardin 1911), *Silba aristella* (Becker) (Schewket 1934), and members of the genus *Earomyia* Zett. (Keen 1958) appear to be the most important. Several species of the genus *Lonchaea* Fallen are recorded as being predaceous on various species of the coleopterous families Curculionidae and Scolytidae (Bedard 1937, Seitner 1922, Taylor 1929).

In North America, no lonchaeids, other than the cone infesting species of the genus *Earomyia*, have been considered as primary pests until the recent discovery of a previously undescribed species of the genus *Dasiops* Rondani infesting apricot fruits in San Diego County, California. Primary injury of apricot fruits by lonchaeid larvae was first found in Ramona, San Diego County, in August of 1945 (Anon. 1959). Determination of the larvae involved was made only to the family level as no adults were reared. The specimens were not retained by the authorities concerned so no comparison can be made with larvae from the current infestation. However, it is quite probable that the same species is involved in both instances as the localities are reasonably close to each other.

The current infestation in Alpine, San Diego County, was brought to the attention of local authorities in 1955 when the degree of infested fruit was estimated at 40%. During the succeeding years and before the initiation of control measures in 1958, the loss as a result of the infestation reached approximately 80% of the crop. Since the initiation of control measures the infestation has been limited to those few fruits remaining on the trees after harvest.

In 1959, another infestation of the same species was found in apricot fruits in Redding, Shasta County, California. This infestation was limited to several dooryard trees whose variety remains unknown (Anon. 1959).

Distribution and Hosts.—*Dasiops alveofrons* McAlpine is distributed through Texas, Arizona, and California, also occurring in Mexico, indicating that this species is apparently limited to the lower Sonoran faunal zone (McAlpine 1961). In California the greater portion of the records are from the southern part of the state, including Los Angeles, Riverside, San Bernardino, and San Diego counties. In the northern part of the state isolated collections have been made in Eldorado, Shasta, and Sonoma counties.

Until the recent discovery of this species infesting apricot fruits, it was known only as a scavenger, having been reared from dead sunflower stems, rotting cactus flowers and walnut husks. Those specimens reared by the authors from walnut husks have always been found in association with the walnut husk fly, *Rhagoletis completa* Cresson. (A survey was made in the area of the infestation in San Diego County for possible native hosts of this species, but none were found.)

The lonchaeid fly has been collected from walnut husks in Sonoma County which is on the northern fringe of the major apricot producing area in California. The occurrence of walnuts throughout most of the apricot producing areas and the concurrent spread of the walnut husk fly increases the possibilities of this insect becoming a major problem of the apricot industry.

Seasonal History and Habits.—The adult fly first appears in the orchard in the middle of May with the first eggs found in the latter part of the same month. The eggs are inserted directly into the flesh of the fruit with as few as five and as many as 34 eggs found in one oviposition site.

This fly will attack both sound and damaged fruits. In sound fruits the favored oviposition site is the seam of the fruit (fig. 1) with the blossom tip also a much favored site (fig. 2). Many times eggs will be found in both areas of one fruit.

In certain varieties, such as the Royal, the skin over the seam of the fruit tends to split forming a fissure which many times extends to the pit. Also, as the fruits ripen birds may peck holes through the flesh which makes access to the area around the pit a simple matter for the female flies. It is under these conditions that eggs have been found in the area between the pit and the flesh.

When eggs are inserted into the seam area of a sound fruit, they are usually located about half the distance to the pit. When the blossom tip is utilized as the oviposition site, the eggs are deposited in the slight depression immediately beneath the epidermis of the fruit. Many eggs are inserted through this depression into the solid flesh beneath.

Upon the hatching of those eggs deposited along the seam, the young larvae migrate to the flesh adjacent to the side of the pit forming a small localized feeding pocket. As the larvae mature the feeding pocket increases in size, ultimately occupying the greater portion of the fleshy part of the fruit. The young larvae emerging from the eggs deposited in the blossom end of the fruit form a localized feeding pocket in the immediate vicinity of the oviposition site and work through the flesh towards the stem end and also ultimately occupying the greater portion of the flesh. As a result of the larval feeding, the flesh of the fruit becomes very moist and pulpy, much resembling that of an overripe fruit. By the time the larvae are mature, the

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[2] University of California Citrus Experiment Station, Riverside.

[3] Department of Agriculture, San Diego, California.

fruit has usually fallen to the ground. The larvae then leave the fruit and pupate in the soil.

This species has been found infesting the Blenheim, Moorpark, Newcastle, Royal and Tilton varieties with the degree of infestation varying with the maturing date of the particular variety. Those varieties, such as the Newcastle, that mature before the Blenheim and Royal are less preferred than the later maturing varieties such as the Tilton and Moorpark, with the Blenheim and Royal varieties also heavily infested.

In the infestation occurring in San Diego County losses have been incurred amounting to approximately 80% of the total crop. As 84% of the bearing apricots in California are composed of the Royal and Blenheim varieties (Friesen 1958), both very susceptible varieties, this insect problem assumes greater significance.

IDENTIFICATION.—The egg of *Dasiops alveofrons* is approximately 1 mm. long by 0.25 mm. wide, slightly curved and tapered towards both ends. When newly laid the egg is translucent but takes on a pearly white color as it matures.

The immature larva is transparent with the tracheae and cephalopharyngeal skeleton quite clearly visible through the body of the insect. As it matures, the larva becomes white in color. At this stage portions of the cephalopharyngeal skeleton remain clearly visible while the tracheae may be obscured. The mature larva reaches a length of approximately 7 mm.

The pupa is 3 to 4 mm. long by 1 to 2 mm. wide with the spiracles protruding at the posterior end. It is light to dark brown in color.

FIG. 1.—Eggs deposited in naturally occurring split in apricot fruit.

FIG. 2.—Eggs deposited in calyx cavity in apricot fruit. Arrow indicates egg inserted deeply into solid flesh of fruit.

The adult is small in size, measuring some 2 to 3 mm. in length. It is a metallic blue-black with clear, bluish-white wings. The female has the long, pointed, sclerotized ovipositor that is one of the characteristics of the family (Collin 1953).

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Ornamental Plants as Virus Reservoirs[1]

FLOYD F. SMITH, *Entomology Research Division*, and PHILIP BRIERLEY, *Crops Research Division, Agric. Res. Serv., U.S.D.A.*

ABSTRACT

Vegetatively propagated perennial ornamental plants are often infected with viruses and serve as virus reservoirs for infection in vegetables and other crops. Common virus sources such as chrysanthemum, gladiolus, dahlia, and lily are widely distributed to many parts of the world. Tomato aspermy, cucumber mosaic, yellow bean mosaic, tomato spotted wilt, and aster yellows viruses are serious because efficient vectors are generally present. Other viruses such as tobacco ringspot and tomato ringspot are also widely distributed in gladiolus and other hosts but lack efficient vectors.

The vegetatively propagated perennial ornamental plants are vulnerable to virus infections, and many such diseases are very difficult to control. These plants are likewise important reservoirs of viruses that damage other crop plants. The seed-propagated annual ornamentals are less significant as virus reservoirs, but occasionally they carry seed-borne viruses; for example, petunia can carry tobacco ringspot virus (Broadbent 1958). Annual ornamentals may serve as build-up hosts. As an example of the latter, the China aster, an annual plant, is highly susceptible to the aster yellows virus. This plant allows the spring-migrant vectors of the six-spotted leafhopper (*Macrosteles fascifrons* (Stal)) to increase the proportion of viruliferous individuals. Thus, such virus-carrying populations inoculate many wild composites and other susceptible perennials that can retain the virus until the following season. Aster yellows is an important disease of the truck crops, carrot, lettuce, and onion (Ken Knight 1943), as well as many ornamentals.

The ornamental plants mentioned here are of course not always virus infected, and those that are do not always encounter effective insect vectors. Explosive virus spread occurs chiefly when a reservoir host grows near a sensitive crop and when efficient vector insects migrate across these plants early in the season at the time the sensitive crop is most vulnerable to virus damage.

ORNAMENTALS OF SIGNIFICANCE.—*Chrysanthemum.*—The ornamentals of most significance as virus reservoirs are the widely grown perennials such as chrysanthemum, gladiolus, dahlia, and lily. Although chrysanthemum stunt has a wide host range among composites (Brierley 1953), this virus has not assumed importance in any plant but chrysanthemum. In contrast the tomato aspermy virus (Brierley *et al.* 1955), which distorts the blooms of some chrysanthemums and is latent in others, is ruinous to tomatoes. Close proximity of the two crops early in the growing season and a spring migration of aphids can lead to complete failure of the tomato crop. Such failures are unknown in commercial tomato production, but they occur in home gardens, where they are commonly misinterpreted as some other disease.

Gladiolus.—Gladiolus is commonly infected with the aphid-transmitted bean yellow mosaic virus (McWhorter *et al.* 1947) which produces mild symptoms in leaves and flowers of many gladiolus cultivars. Beans in gardens or in commercial fields are damaged whenever they grow in proximity to gladiolus. This relationship of the two crops has been noted also in Holland (Van der Want 1954).

Cucumber mosaic virus (CMV), first noted in gladiolus in Tasmania (White 1946), has become a major disease of the plant in sections of the United States. The white streaking in leaves and white-striped flower breaking are distinctive, but transmission by aphids, delayed expression of symptoms, and the difficulty of roguing small stock plants permit the disease to increase. Nevertheless successful control by roguing is reported from Oregon (McWhorter 1957). Aphids can move cucumber mosaic virus from infected gladiolus to susceptible crops such as tomato, pepper, and spinach, but gladiolus is of chief significance as a distributing host of CMV. Inasmuch as gladiolus corms are shipped in quantity all over the world, they are likely to spread CMV into every area that is home for a susceptible plant and an aphid vector. Other hosts of CMV could do likewise, but few ornamentals are moved as widely or in such bulk as gladiolus.

Gladiolus is also subject to aster yellows infection. In the humid East this virus disease is a mere curiosity, occurring in trace amounts of no economic importance. In western agriculture where irrigation is practiced gladiolus is occasionally badly damaged by aster yellows (Magie *et al.* 1952). If infected after flowering, the plants show no symptoms until the following year (Smith & Brierley 1953) when they lose apical dominance and develop weak "grassy" shoots from each bud. This disease is so virulent that the plants die in the second season and approach self-elimination. Because normal growth of gladiolus is not especially attractive to the leafhopper vector and the weak grassy growth of diseased plants is even less so, gladiolus assumes little importance as a distributor of western aster yellows virus into Eastern United States and elsewhere.

Both tobacco and tomato ringspot viruses are common in gladiolus. As systemic invaders of the plant, the ringspot viruses are also widely distributed in gladiolus, but they are less likely to invade other crops than other pathogens such as bean yellow mosaic virus (BYV) and CMV which have efficient vectors.

Dahlia.—Dahlias are considered the chief means of disseminating tomato spotted wilt virus (TSW) in the Pacific Northwest (McWhorter 1941). Symptoms in dahlia are ring patterns or mosaic-like mottling with some rings. Tomatoes and most truck crops are damaged by TSW, which is moved by thrips. Dahlias are also considered an important reservoir of TSW in England (K. M. Smith 1933) and in Belgium (Roland 1948), but other perennial ornamentals such as calla (*Zantedeschia* spp.) (Tompkins & Severin 1950), and canterbury bells (*Campanula*) (Noordam 1952) may also serve. Holmes-Smith (1934) found tomatoes more subject to TSW when grown in

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mixed nurseries near young ornamentals, especially vegetatively propagated perennials. Dahlia proved the chief source but nasturtium (*Tropaeolum*) and other ornamentals also served as reservoirs.

Dahlias also carry cucumber mosaic virus, often without symptoms, in Belgium (Roland 1948), England (K. M. Smith 1952), and the United States. Although CMV has a very extensive host range, the dahlia is important as a reservoir because it is nearly symptomless when infected and is vegetatively propagated and readily shipped to many parts of the world. Lilies (*Lilium*) are in the same category, inasmuch as many species can carry CMV without symptoms, and the bulbs are shipped widely.

PLANTS OF LESS IMPORTANCE.—*Ornamentals.*—Ornamental plants are of negligible importance as reservoirs of some of the major viruses. Although several ornamentals are susceptible to curly top, these serve no essential part in overwintering dissemination or transport of this virus (Severin 1934). A few ornamentals of the potato family are subject to tobacco mosaic virus, but they are of incidental importance. Likewise the ornamental hosts seem to fill no essential role in distribution of the soil-borne viruses of tobacco rattle, tomato blackring, and tobacco necrosis, unless tulip (K. M. Smith 1955) and primula (Price *et al.* 1950), among the few suspects to be systemically invaded by tobacco necrosis virus, be regarded as important in transport of this virus.

In Idaho, Ken Knight (1943) considered flower borders as one source of aster yellows inoculum for vegetable seed crops such as carrot, lettuce, and onion. In Bulgaria, Kristova (1959) reported that virus diseases of vegetables increased after flower growing was introduced and expanded on the same farms. She then listed some flower hosts of TSW and CMV. In Germany, Uschdraweit & Valentin (1959) screened 6000 plants, mostly perennials, for winter hosts of important viruses. They found 283 species infected, 173 with CMV, 96 with a virus of primroses. Up to 25% of the infected ornamentals yielded tobacco rattle virus, and 10% of them ringspot and turnip viruses. Wild plants were less commonly infected than ornamentals. Infected perennials generally survived the winter without injury. In another study the same workers found 8% of 500 plants in Berlin municipal parks and gardens infected with tobacco rattle virus (1956). These ornamentals appear to play a significant role as virus reservoirs in Europe as well as in the United States.

Truck Crops.—It may be asked whether truck crops present a virus hazard to ornamental plants. For two reasons this hazard is less than that of the ornamentals to truck crops. There are few vegetatively propagated perennials among truck crops, and truck crops are now commonly sprayed. Sweet potatoes appear to have viruses peculiar to the crop but occasionally harbor tobacco mosaic (Elmer 1960). Irish potatoes were formerly important for build-up of vector aphids that could bring about an explosive spread of viruses in such crops as Easter lily. Modern insecticides now commonly applied to potatoes minimize this hazard.

Areas long planted to truck crops are notorious as danger areas for CMV. Easter lilies regularly become infected with CMV if planted in such truck-crop areas. Formerly vegetables such as pepper, tomato, and cucumber served as build-up hosts for CMV and for its aphid vectors, thus steadily increasing infection in perennial wild plants and weeds that can overwinter this virus. The build-up is now less likely to continue when truck crops are sprayed, but the stage is set, and truck crop areas remain a menace to CMV-sensitive crops.

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Adjuvants Increasing the Residual Activity of Phosdrin[1]

H. E. ALLER and J. E. DEWEY, *Department of Entomology, Cornell University, Ithaca, New York*

ABSTRACT

Twenty-three materials were screened as potential adjuvants to increase the residual effectiveness of Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate) on fourth instar larvae of Mexican bean beetle (*Epilachna varivestis* Muls). Phosdrin residues 24, 48, and 72 hours old were infested and mortality observed after periods of 24 and 48 hours. The gross performance of each concentration of each material was compared with the performance of Phosdrin standard treatments.

Six materials appeared promising. They are Experimental Plyac® (polyethylene formulation), Prorex-1® (high paraffin petroleum oil), Dri-Die 67® (silica aerogel), Nuchar C-115N® (active carbon), Cellosize® WP-4400 (hydroxyethyl cellulose polymer), and Methocel® MC-4000 cps. (methyl cellulose polymer). Of these, the last two were outstanding. At 2%, both of these water soluble cellulose ether polymers more than doubled the residual efficacy of Phosdrin. Methocel alone was found toxic, and thus may not act as an adjuvant in this instance.

Several toxicants available today have a short period of residual effectiveness. Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate), DDVP, and Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) are typical examples. Short residual effectiveness is desirable in an insecticide applied immediately before harvest so that no residue will be present at harvest. Due to the short life of such compounds, several applications may be necessary to provide adequate protection. In such instances, longer residual activity may be advantageous. Improvement of the residual efficacy of insecticides may sometimes be accomplished by the inclusion of suitable adjuvants in the formulation.

It was the purpose of this study to screen those materials which might serve as adjuvants to increase the residual effectiveness of Phosdrin against fourth instar larvae of the Mexican bean beetle, *Epilachna varivestis* Muls. Although an adjuvant may act in more than one capacity, no attempt was made to ascertain the type of action of the materials. The 23 materials screened represent but a small portion of the possibilities.

MATERIALS AND METHODS.—Prior to screening, a dosage mortality curve was established for a 24-hour-old Phosdrin residue on which larvae were confined for an additional 24 hours. Concentrations of Phosdrin were prepared by adding the required volume of toxicant to tap water containing Triton X-100®[2] at 0.3% (v/v). The average mortality for each concentration was plotted on log-probability paper. The LD_{50} concentration from an eye fitted line was 0.0135% (v/v). This concentration was used in all the screening operations and in the treatment standards throughout the study.

Each formulation containing Phosdrin and adjuvant was prepared by first making up the Phosdrin solution in the same manner as for the mortality curve. Weighed amounts of each adjuvant were added to the toxicant solution. When the adjuvant was not readily miscible with water, acetone at 10% (w/v) of the total mixture was used. Adjuvants were used at concentrations of 1% and 10% wherever possible. A list of the tested materials with their chemical designations is presented in table 1. In early tests mortalities in the untreated check seldom approached 5%. No further provision for untreated checks was made in order to make use of the facilities and larvae available.

Fordhook 242 lima beans germinated in sterile vermiculite were used as test plants since they were not wilt-prone after the roots were excised. After excision of cotyledons and roots, seedlings with large primary leaves were individually inserted in commercial orchid tubes. To retard bacterial growth, a 0.005% sodium hypochlorite solution was used in the tubes. Plants were dipped in the Phosdrin solution in groups of three. To insure complete coverage, three 3-second dips were made in rapid sequence. A minimum of 18 plants was used for each concentration. After dipping, the treated plants were placed in constant temperature room held at 80° F. until the residues were 24 hours of age. Water lost from the tubes because of plant transpiration was replaced as needed.

Before infestation, the tubes were inserted bottom first through a hole bored in the bottom of plastic cups 4 inches in diameter and either $1\frac{1}{2}$ inches or 3 inches high. The rubber cap on the tube seated the tube securely. A thin film of petrolatum was applied to the rim of each cup to prevent larval escape. Cups containing plants were placed in supporting racks in a 75° F. constant temperature room and then infested. Each concentration was

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[2] Rohm & Haas Company, Philadelphia, Pa.

Table 1.—Materials screened as possible adjuvants for increasing residual effectiveness of Phosdrin against fourth instar Mexican bean beetle larvae and toxicity rating index.[a]

Compound and Source	Chemical Definition	Concentration (%)	Residual Age Indices 24-hr.	48-hr.	72-hr.	Overall Index	Phytotoxicity Rating
	A. Inefficient combinations						
Glyodin® Union Carbide, N. Y.	2-heptadecyl glyoxalidine acetate	1.00	0.8	0.8	0.5	0.8	
		10.00	.8	1.3	.5	.9	
Crag Fly Repellent® Union Carbide, N. Y.	Butoxypolypropylene glycol	0.02	.3	1.1	.5	.6	
		0.20	.8	.4	.4	.6	
Thiokol® ZM-198 Thiokol, Trenton, N. J.	Polysulfide polymers	1.00	.4	.8	.4	.5	Moderate
		5.00	.3	.0	.1	.1	Severe
Thiokol® ZM-234	"	1.00	.5	.7	.6	.6	Moderate
		5.00	.2	.2	.0	.1	Severe
Aerosol® OT—100% American Cyanamid, N. Y.	Sodium dioctyl sulfosuccinate	0.80	.3	.3	.4	.3	Moderate
Multifilm® X-77 Colloidal Products, Sausalito, Calif.	Mixtures of polyethylene glycols and other chemicals	1.00	1.1	.2	.4	.6	Slight
		10.00	1.6	1.2	1.3	1.4	Severe
Multifilm® P-926-A-MO	"	1.00	1.3	.9	.6	1.0	
		10.00	1.3	.9	.7	1.0	
Multifilm® R-139	"	1.00	.6	.8	.6	.7	
		10.00	.7	1.3	.6	.8	
Multifilm® R-163	"	1.00	.5	.3	.1	.4	
		10.00	.5	.6	.7	.6	Moderate
Vancide Sticker® R. T. Vanderbilt, N. Y.	Synthetic rubber emulsion	1.00	.7	.8	1.0	.8	
		10.0	1.1	.8	.5	.9	
Glycerin	1,2,3-propanetriol	0.02	1.0	.8	.6	.8	
		0.20	.8	.5	1.2	.8	
Carbowax® 200 Union Carbide, N. Y.	Polyethylene glycols	1.00	.6	.7	.4	.6	Slight
		10.0	1.1	1.1	1.1	1.1	Moderate
Carbowax® 300	"	1.00	.6	.5	.3	.4	Slight
		10.00	1.4	2.2	1.1	1.5	Severe
Aroclor® 1260 Monsanto, St. Louis, Mo.	Resinous chlorinated bi- and terphenyls	0.10	.6	.3	.4	.5	
		1.00	1.5	.5	.6	1.0	
Aroclor® 4465	"	0.10	.7	.2	.1	.4	
		1.00	1.0	.4	.1	.6	
Aroclor® 5460	"	0.10	.6	.3	.2	.4	
		1.00	1.1	.9	.7	.9	
Acetone		10.00	.9	.4	.8	.7	
Plyac® Allied Chemical, N. Y.	Polyethylene formulation	1.00	.8	.9	1.0	.9	
		10.00	.6	.1	1.2	.6	
	B. Combinations exhibiting beneficial effects						
Plyac® Experimental	Polyethylene formulation	1.00	.8	1.2	1.3	1.0	
		10.00	1.0	1.5	2.0	1.4	
Prorex®-1 Socony Mobil Oil, N. Y.	High paraffin petroleum oil	0.02	1.3	1.1	2.5	1.6	
		0.20	1.0	1.4	1.2	1.2	
Dri-Die Insecticide 67® Davidson, Baltimore, Md.	Silica aerogel	0.02	1.2	1.2	1.9	1.4	
Nuchar® C-115N West Virginia Pulp & Paper, New York	Active carbon	0.02	1.1	.9	1.8	1.2	
		0.20	1.6	2.3	2.0	1.9	Slight
Cellosize® WP-4400 Union Carbide, N. Y.	Hydroxyethyl cellulose polymer	1.00	.8	1.8	1.2	1.2	
		2.00	2.1	—	3.5	2.6[b]	
		(2.00)[c]	(.08)	—	(.04)	(.07)[b]	
Methocel® MC-4000 cps. Dow, Midland, Mich.	Methyl cellulose polymer	1.00	1.3	2.1	1.7	1.6	
		2.00	2.1	—	3.8	2.7[b]	
		(2.00)[c]	(1.2)	—	(1.3)	(1.3)[b]	
	C. Phosdrin and proprietary formulations						
Phosdrin technical Shell Chemical, N. Y.	(See text)	0.0135	1.0	1.0	1.0	1.0[d]	
Phosdrin emulsible Shell Chemical, N. Y.	"	0.0164[e]	1.0	1.3	1.7	1.2	
Phosdrin 80% paste Shell Chemical, N. Y.	"	0.0164[e]	1.1	1.2	1.1	1.2	

[a] The overall index value is derived from the 24-, 48-, and 72-hour residue index values.
[b] Due to missing plots, a correspondingly altered overall standard average set at 1.0 was used to calculate these overall index values.
[c] Material tested without Phosdrin.
[d] Base values for standard treatment used to derive values, excepting those marked[b].
[e] 0.0164% (w/v) = 0.0135% (w/v).

Table 2.—Mortality of fourth instar larvae of Mexican bean beetle exposed to residues of Phosdrin "standards" applied at 0.0135% (v/v).[a]

Age of Residue (hours)	Observation Period (hours)	No. Larvae	Mortality (%)
24	24	617	40.9
	48	617	55.1
48	24	461	18.0
	48	461	40.4
72	24	609	16.1
	48	609	36.0

[a] Percentages are weighted averages derived from all tests.

replicated three times. Each replicate contained 20 larvae which were reared by conventional methods in the greenhouse using Tendergreen beans as food plants. Fluorescent lighting was maintained throughout the experiment.

After exposure for 24 hours, the larvae were jarred from each cup, prodded, and assumed dead if unable to move at least one body length with coordination. After adding a fresh plant, treated at the same time as the one consumed, all larvae were returned to the bottom of the cup for 48 hours cumulative exposure. Thus, for each treatment, observations were made after 24- and 48-hour exposure periods for residues 24, 48 and 72 hours old.

For comparative purposes, daily determinations of Phosdrin "standards" were run using the LD_{50} concentration. The treatment and observation periods were the same as in the adjuvant treatment mixtures. Mortality data were not subjected to standard statistical methods since differences in performance were of sufficient magnitude to be readily apparent and comparable by the following methods. To facilitate comparisons, daily determinations of Phosdrin standards were pooled to form weighted averages for each exposure period within each residue treatment (table 2).

The numerical average of all the per cent mortalities of the standards stated in table 2 was used as the divisor for similarly derived overall numerical averages for each concentration of each adjuvant screened. An arbitrary rating system was adopted to simplify presentation, whereby the overall average of the per cent mortalities of the standards was assigned an "overall index value" of 1.0. When this average was used as a divisor for adjuvant-Phosdrin treatment, the resultant "overall index values" appear in terms of multiples of the standard treatment. For example, the "overall index value" for Glyodin used at 1% (table 1) was 0.8, or 0.8 times as effective as the standard treatment. This type of presentation combined the effects of residual age and speed of kill and was used to compare gross performances of the adjuvants. For completeness, separate indices were calculated for each age of residue by the same process. When phytotoxicity occurred, it was rated as being slight, moderate, or severe.

RESULTS.—It was convenient to group the candidate adjuvants into two groups, based on the overall rating scheme described above. The first group generally consists of adjuvant-Phosdrin combinations whose overall rating indices were 1.0 or less. However, some combinations with higher indices were included if phytotoxicity occurred. This group will not be discussed further. The second group consists of combinations which exhibited beneficial effects to a degree warranting further investigation. Index and phytotoxicity ratings are presented in table 1.

Six materials appeared promising in combination with Phosdrin. Dri-Die 67, Prorex-1, Nuchar C-115N, and the Experimental Plyac sustained or improved the residual effectiveness throughout the experiment. Prorex and Nuchar also inhibited feeding on the 24-hour residues. Nuchar and Dri-Die produced what may be considered an objectionably visible residue. Nuchar was also difficult to wet and suspend in water.

The cellulosics, Cellosize and Methocel, were clearly superior adjuvants in initial screening. Unexpectedly good results prompted retesting these two materials at 2% with and without Phosdrin. As adjuvants in combination with Phosdrin, both compounds proved more than twice as effective as the standard treatment. Without Phosdrin, Cellosize was virtually nontoxic, whereas Methocel at 2% produced a sustained residual toxicity exceeding that of Phosdrin. Both cellulose polymers as adjuvants at 2% concentration gave almost complete control.

Proprietary formulations of Phosdrin performed better than the standard treatment. They were recognized as separate entities and therefore were not placed in either of the two groupings.

CONCLUSIONS.—Six superior materials were found by screening to indicate that retention of Phosdrin's residual effectiveness may be accomplished by the inclusion of a suitable adjuvant. Methocel, Cellosize, and Experimental Plyac are dry-film-formers which apparently either retarded volatilization or contributed to increased deposition of the toxicant. Methocel possessed some degree of toxicity and for this reason it is questionable if the term adjuvant is applicable. Prorex-1 may also act to some degree as a primary insecticide, but was considered to be a wet- or oil-film-former. Nuchar C-115N and Dri-Die 67 probably associated with Phosdrin in a sorptive capacity.

Compounds conventionally utilized as spreaders and/or stickers did not appear to enhance the effectiveness of Phosdrin residues. The high concentrations employed in the screening of such materials may have contributed to toxicant loss caused by excessive run-off. Hence the results obtained may not be truly indicative of the full potential of any compound.

Evidence of improved effectiveness caused by formulation with suitable adjuvants indicates the need of examination of existing compounds exhibiting film-forming or sorptive capacities. Such compounds appear to be well suited for combination with volatile toxicants, despite their lack of prior usage in this capacity.

Acaricidal Activity of Cellulose Polymers[1]

H. E. Aller and J. A. Naegele, *Department of Entomology, Cornell University, Ithaca, New York*

Abstract

Four water-soluble etherified polymers were found to give good control of a demeton-resistant laboratory strain of the two-spotted spider mite (*Tetranychus telarius* (L.)). They were Cellosize® (hydroxyethyl cellulose) WP-4400, Methocel® (methyl cellulose) MC-4000 cps., and CMC® Cellulose Gum (sodium carboxymethyl cellulose) 7L, and Ceron® (hydroxypropyl starch) N-48. Mortality, which precedes dry film formation common to all these compounds, is characterized by derangement of the internal components of the mite. This mode of action is evidenced by all four polymers at the higher concentrations employed (1.0% and 0.5%) and is independent of adhesion of the mite to the leaf. Control is essentially limited to motile forms.

Spray applications of Cellosize and Methocel (4 lb. per 100 gallons) on nine rose varieties in commercial greenhouses, effectively reduced mite populations. Phytotoxicity on roses consisted chiefly of distortion attributable to mechanical restrictions imposed by the residual film on developing foliage. However, repeated applications were noted to cause premature abscission and marginal leaf burn on some varieties. Foliar and floral necrosis was observed on a few conservatory plant species treated with Cellosize.

All four compounds are commercially produced and possess an extremely low mammalian toxicity. Dispersion in water requires particular attention. Further investigation regarding the nature of these compounds is being undertaken.

Several materials have been used to kill mites by mechanical means. Among these are flour paste (Parker 1913), bill-posters' cement (Hutson 1936), gelatin (Steiner 1939) and polybutenes (Fisher 1959). Film production is characteristic of some compounds commercially employed as acaricides and prompted investigation of the cellulose derivatives as control agents for the two-spotted spider mite (*Tetranychus telarius* (L.)). Although some film-forming cellulosics have been found to be effective in increasing the residual life of Phosdrin (Aller & Dewey 1961), no prior utilization of these compounds as acaricides is known to the authors.

Materials and Methods.—All the compounds employed in this study are water soluble, colloidal, long chain polymers comprised of hexose units. The three major cellulose ethers are commercially available in a wide range of viscosities or chain lengths. Specifically the compounds used were Cellosize® WP-4400, Methocel® MC-4000 cps., and CMC® Cellulose Gum 7L. Ceron® N-48, an etherified starch derivative was also tested.[2] All are produced in dry form and possess extremely low mammalian toxicity.

Laboratory screening consisted of dipping replicates of mite-infested Fordhook 242 lima bean plants in polymer dispersions. A strain of two-spotted spider mite highly resistant to demeton was used. Three 3-second dips in rapid succession were made to insure adequate coverage. Concentrations employed were in arithmetic dilution from a maximum 1% (w/v), so that each concentration was halved to form the next dilution of the series. Cellosize, CMC, and Ceron were readily soluble in cold water. Methocel required initial dissolution in hot water. The powder was slowly added to the water while rapidly stirring so as to avoid agglomeration. Triton X-100[3] at 0.03% (w/v) was employed at all concentrations as a surfactant.

Mortality counts were made 24 hours after treatment and the per cent mortality corrected by Abbott's formula (Abbott 1925).

Results.—*Laboratory.*—The results of the laboratory studies are presented in table 1. Films formed by Cellosize dips were adequate to cement mites to the leaf and thereby accomplish kill. However, treated mites had clearly undergone radical internal disturbances. Microscopic examination of mites immersed in drops of 0.5% Cellosize on a slide indicated that these disturbances occurred prior to film formation. Within minutes after immersion the internal tissues drew away from the integument, chiefly in the dorsal region. This was accompanied by a lateral expansion of the combined hind gut and excretory organ. The resultant compression caused rearrangement of the ventriculus and its contents. The ventriculus was displaced to either a posteriad or anteriad position or ruptured laterally extruding food balls into the leg cavities. Concurrently, eye-spot pigments exhibited streaming, usually posteriorly. Moribund movements included pulsation of fecal matter in the hind gut and spasmodic operation of the pharyngeal pump. These movements were accompanied by abnormal and erratic "churning" within the ventriculus.

Final drying of the film often caused rupture of the integument in the region of the genital opening. Partial vacuolization of the appendages and body cavity occurred, rendering the mite a doughnut-shaped body cemented to the treated surface. This dorso-ventral flattening was apparently restricted to concentrations in the range of 0.5% and 1.0% for the starch and cellulose polymers. Acaricidal activity was limited largely to motile forms, with the majority of eggs and quiescent stages surviving.

Field.—A number of conservatory plants were sprayed with Cellosize WP-4400 to determine plant sensitivity and ease of application. An initial application of 1% was applied with a 25-gallon Bean electric sprayer at 200 p. s. i. pressure using twin nozzles with number 4 discs. The solution was quite viscous and the spray very coarse. Reapplication was made 5 days later using 0.5% Cellosize applied by a 1.5-gallon hand sprayer with a finer nozzle. This application was more uniform but

[1] This study was partially supported by a research grant from Roses Inc. Partial cost of publication of this paper was met by Roses Inc., East Lansing, Mich. Accepted for publication February 27, 1961.

[2] The chemical names of the proprietary compounds mentioned in this paper are given in the paragraph preceding "References Cited."

[3] Rohm & Haas Company, Philadelphia, Pa.

Table 1.—Mortality[a] of the two-spotted spider mite (demeton-resistant laboratory strain) 24 hours after dipping infested bean plants in dispersions of cellulose and starch derivatives.

Concentration (%)	Cellosize WP-4400		Methocel MC-4000 cps.		CMC-7L		Ceron N4S	
	Mortality (%)	No. Mites	Mortality (%)	No. Mites	Mortality (%)	No. Mites	Mortality (%)	No. Mites
1.0000			99.2	1,779	81.7	638	86.8	456
0.5000	96.8	2,154	97.4	2,105	80.7	419	76.2	352
0.2500	95.4	2,182	96.2	307	47.9	256		
0.1250	86.5	346	79.1	593	19.2	233		
0.0625	13.9	587	41.6	553	9.8	195		
0.0313	9.0	472			8.0	199		

[a] Calculated according to Abbott's formula (Abbott 1925).

limited agitation allowed agglomerate formation which periodically resulted in a clogged nozzle. Ideally, uniform dispersion is best obtained by allowing a mixture with half the required water to stand 4 hours prior to adding the remaining water for application. However, in practice, spray tank agitation with the full complement of water for 15 minutes was utilized in place of standing. The resultant mixture may thicken during application but apparently this does not adversely influence spraying, deposition, and acaricidal effectiveness.

All plants retained a high gloss film 1 week after the initial application. Some fast growing plant tissue ruptured the film but the resultant film peelings, or for that matter all deposits, were removable by several syringings of water by hose. A complete phytotoxicity report is beyond the scope of this paper; however, blossoms of *Saintpaulia*, *Oxalis*, and *Cattleya* orchid were quite sensitive, exhibiting severe necrosis. Foliar necrosis is restricted largely to tender and young monocotyledenous plants such as cultivated *Oxalis* varieties, and consists of either localized necrotic spots or marginal leaf burn. Specimens from the banana family, Musaceae, exhibit moderate to severe chloranemia. African violet (*Saintpaulia ionantha*) foliage was un.lamaged, as were the majority of the species treated.

Application of Cellosize QP-4400 was initiated in commercial greenhouses producing cut roses. (Cellosize QP-4400 differs from WP-4400 only in being somewhat more dispersable in water, thereby avoiding agglomerate formation.) Stock solutions were avoided since gelation occurred. The material was added slowly to the full amount of water required and dispersion facilitated by tank agitation for 15 minutes prior to application. Cellosize was used at 4 pounds per 100 gallons supplemented with 4 ounces of either Triton X-100 or NNO.[4]

A total of more than 58,000 plants encompassing nine rose varieties have been treated with Cellosize. The varieties were: Sterling Silver, White Butterfly, Carol Amling, Pink Garnette, Garnette, Better Times, Jingles, Castanet, and Rosenelfe. Pre- and posttreatment field ratings were generally obtained by sampling leaves showing mite injury from each plot and assigning each leaf a value. The rating scale employed was: 0=no mites alive, 1=1 to 2 mites, 2=3 to 10 mites, 3=11 to 100 mites, and 4=100 +mites alive. The numerical average of the values from 25 leaves established the field rating for each plot. No plot contained less than 150 rose plants.

To facilitate presentation, ratings were summarized by averaging over locations, plots, and varieties. Essentially, the degree of mite control was similar in all plots. The average pretreatment field rating for one group of 16 plots was 1.50. The average rating 1 to 4 days after treatment was 0.32. Another group of four plots was rated 1.20 prior to treatment and 0.28 five to seven days after treatment. This degree of field control is comparable to that of organophosphates on nonresistant populations.

Plant injury was occasionally produced by Cellosize film restricting expansion of developing plant tissues. In floribunda roses, such as Garnette, young leaflets were unable to expand normally without distortion unless the film is subsequently softened by overhead misting. In hybrid teas, such as Better Times, localized deposits on very young foliage sometimes produced unequal growth and distortion. Premature abscission of old foliage or "ripening" as well as some marginal leaf burn has been observed on some varieties after repeated applications.

To date, Methocel has been commercially applied to only one plot consisting of White Butterfly. Employed at the same rate as Cellosize, it requires dissolution in hot water, an inconvenience limiting its usage to locations having the proper facility. Mite populations were reduced from a field rating of 1.60 to 0.41 when rated more than 2 weeks after treatment.

Discussion.—Acaricidal activity of cellulose and starch polymers was demonstrated in laboratory screening. Adhesive films were formed by all compounds, but at least with the higher concentrations employed, control apparently is other than mechanical in nature. Marked internal disruption of the mite tentatively indicates either a high water demand, or selective sorption of some essential component(s) within the mite during the sol-gel transition. The dried film had no observed residual activity. Mode of action of these compounds is currently under further investigation.

Field application of Cellosize and Methocel on roses reduced mite populations as effectively as conventional toxicants. Sustained control requires a spray program correlated with the life cycle of the mite. Low mammalian toxicity eliminates application hazards and suggests possible utilization on food crops. Incidental observations indicated contact insecticide potential for control of soft-bodied insects such as aphids and motile stages of scale insects.

[4] Atlas Powder Co., Wilmington, Del.

Chemical definitions of materials bearing proprietary names referred to in this paper are:

Cellosize	(hydroxyethyl cellulose)
Methocel	(methyl cellulose)
CMC	(sodium carboxymethyl cellulose)
Ceron	(hydroxypropyl starch)

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Intracellular Distribution of TPN-Isocitric Dehydrogenase Activity in Susceptible and Insecticide-Resistant Strains of *Aedes aegypti*[1]

M. R. V. MURTHY and DON W. MICKS,[2] *Laboratory of Medical Entomology, Department of Preventive Medicine and Public Health, University of Texas Medical Branch, Galveston*

ABSTRACT

Spectrophotometric determinations were made of TPN-isocitric dehydrogenase activity in the mitochondria and soluble fractions of one susceptible and two insecticide-resistant strains of larvae of *Aedes aegypti* (L.). It was found that the enzyme activity of the soluble fraction was approximately the same in all three strains, but mitochondria showed appreciable differences in activity. The soluble fraction contained from 10 to 40 times the activity of mitochondria depending upon the strain.

DDT added at a concentration of 1.1×10^{-4} M inhibited the TPN-isocitric dehydrogenase activity in the mitochondria of the S and DDT-R strains, whereas it increased the enzyme from the corresponding fraction in the DL-R strain. The same insecticide increased the soluble TPN-isocitric dehydrogenase of the S and DDT-R strains and inhibited it in the DL-R strain.

Dieldrin at a concentration of 1.1×10^{-4} M behaved in a manner similar to DDT on the TPN-isocitric dehydrogenases of the DL-R strain by increasing the mitochondrial enzyme and inhibiting the soluble enzyme

The effects of DDT and dieldrin, and the possibility of biochemical differentiation of TPN-isocitric dehydrogenases derived from the two cellular locations are discussed.

Much of the work on the biochemistry of insecticide resistance has been conducted with the adult house fly, *Musca domestica L.*, in which DDT resistance is linked with the detoxication of the insecticide by the enzyme DDT-dehydrochlorinase. There is also evidence that DDT exerts an effect upon specific enzyme systems. For example, Sacktor (1950) observed that DDT-resistant house flies contained on the average, 50% more cytochrome oxidase activity than those of a susceptible strain. He noted further (1951) that the pupae of the same resistant strain had less of the enzyme than the susceptible strain. Later, Perry & Sacktor (1955) showed that although some strains differed in cytochrome oxidase activity, no distinctions could be made in this respect between all resistant and susceptible strains. Pant & Dahm (1957) found no effect of DDT on the cytochrome oxidase of house flies *in vivo*, but the succinic dehydrogenase activity was inhibited. Barsa & Ludwig (1959) reported that in homogenates of *Tenebrio* and *Musca*, succinic dehydrogenase was the only one of the nine dehydrogenases tested that was inhibited by DDT.

The mechanism of DDT resistance in mosquitoes is still an open question as to whether it is related, as it is in the house fly, to its detoxication by dehydrochlorinase to DDE. It was found by Brown & Perry (1956) that DDT-resistant larvae of *Aedes taeniorhynchus* (Wied.) produced much DDE, and the susceptible larvae virtually none; however, DDT-dehydrochlorinase as characterized from resistant house flies could not be demonstrated. The mechanism of dieldrin resistance remains a mystery even in the house fly (Brown 1960).

The present work is part of a study to determine the effects of insecticides on the oxidative enzymes of susceptible and insecticide-resistant strains of larvae of *Aedes aegypti* (L.). Some experimental evidence is submitted to show that TPN-isocitric dehydrogenase activity is present in both the mitochondrial and supernatant fractions of the larvae, that the two enzymes behave differently in the presence of DDT and that the mitochondrial enzyme activity is different in the strains studied.

MATERIALS AND METHODS.—Fourth-instar larvae of three strains of *Aedes aegypti* were used in all experiments. One strain (UTMB) was susceptible to both DDT and dieldrin (the respective LC_{50}'s being 0.5 p. p. m. and 0.1 p. p. m., respectively) and will be referred to hereafter as the S-strain. The other two strains were insecticide-resistant. One was the DDT-resistant (Trinidad) strain (LC_{50}'s for DDT and dieldrin—2.5 p. p. m. and 0.1 p. p. m., respectively) which will be termed the DDT-R

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strain. The other was the dieldrin-DDT resistant (Puerto Rico) strain, the LC_{50}'s of which were dieldrin—2.5 p. p. m. and DDT—1.5 p. p. m. This will be referred to throughout as the DL-R strain.

DDT (p,p′) and dieldrin (purified HEOD) were used in acetone solutions. It was first determined experimentally that acetone in the final concentration employed did not have any effect on the isocitric dehydrogenase activity of the mitochondrial and supernatant preparations when measured under the above conditions. When diluted with water the acetone solution of DDT at high concentrations tended to give a slightly opalescent solution which increased progressively with time and reached a plateau at the end of 30 minutes. The DDT solutions were therefore added to the reaction mixtures and maintained at the experimental temperature of 24° C. for 30 minutes prior to starting the reaction with the addition of the enzyme.

The larvae were harvested during the fourth stage and washed several times with double distilled water. After blotting out the moisture, they were weighed quickly and immobilized in ice. They were then ground gently in an ice-cold glass homogenizer with 5 volumes of an extraction medium containing 0.33M sucrose, 0.6% bovine serum albumin and 10^{-4}M Ethylene diamine tetra-acetate (EDTA) adjusted to pH 7.4 with KOH. The homogenates were centrifuged at 600g in a Spinco ultracentrifuge to remove the unbroken cells and cell fragments, and the mitochondria were sedimented by centrifuging at 8000g for 7 minutes. The pellets were washed twice by recentrifugation of the re-suspended material. The final mitochondrial suspension was adjusted in the same extraction medium so that 1 ml. represented 0.5 gm. of the original larvae. The first supernatant fraction obtained in the same experiment was centifuged at 105,000g for 90 minutes to remove the submicroscopic particles. The soluble portion was carefully decanted and then diluted 1:4 with 0.33 M sucrose to give a final solution, 1 ml. of which was equivalent to 0.05 gm. of the larvae.

When the soluble fraction was prepared independently, the larvae were homogenized in 5 volumes of the extraction medium and centrifuged at 105,000g for 90 minutes. The supernatant was decanted and diluted with 0.33 M sucrose as described above. All manipulations were carried out at 0° C.

Determination of isocitric dehydrogenase activity was carried out spectrophotometrically at 24° C. by following the rate of reduction of TPN (triphosphopyridine nucleotide) on addition of the enzyme source. The reaction mixture contained 3.3×10^{-2}M Tris (trishydroxymethylaminomethane) buffer pH 7.4, 10^{-4}M TPN, 0.66×10^{-3}M $MnSO_4$, 5.5×10^{-2}M nicotinamide, a suitable level of the enzyme preparation and 1.33×10^{-3}M dl-isocitrate, the total volume being made up to 3 ml. with double distilled water. The blank contained all reagents except isocitrate. The reaction was initiated by the addition of the enzyme. Whenever mitochondria were used as the enzyme source, 0.83×10^{-4}M KCN was also included in the reaction mixtures. On addition of enzyme, the reaction was followed by measuring the increase in optical density, at 340 mμ at 30 second intervals in the Beckman Model DU spectrophotometer. The enzyme activity was calculated as micromoles of TPN reduced per 100 mg. of the wet weight of original larvae per hour, using the molecular extinction coefficient of 6.22×10^6 sq. cm. $\times$Mole^{-1} for TPN at 340 mμ (Horecker & Kornberg 1948). The linear part of the reaction rate curve in the first few minutes was used for calculating the enzyme activity.

Table 1.—Effect of DDT[a] and dieldrin[a] on the TPN-isocitric dehydrogenase activity of cell fractions from DDT-dieldrin susceptible (S), DDT-resistant (DDT-R) and dieldrin-resistant (DL-R) strains of larvae of *A. aegypti*.[b]

	S	DDT-R	DL-R
Mitochondria	0.22±0.005	0.80±0.008	0.50±0.006
Mitochondria+DDT	0.15±0.003	0.56±0.007	0.61±0.006
Mitochondria+dieldrin	—	—	0.68±0.011
Soluble fraction	9.97±0.082	10.02±0.103	9.87±0.102
Soluble fraction+DDT	11.25±0.210	12.25±0.265	5.98±0.233
Soluble fraction+dieldrin	—	—	6.17±0.264

[a] 1.1×10^{-4} M.

[b] Values represent specific activities expressed as the mean of six replicates plus or minus their standard errors.

The reaction mixture contained nicotinamide to prevent enzymic destruction of pyridine nucleotides by nucleotidases taking into consideration that mitochondria from adults of *A. aegypti* appear to have appreciable DPN-ase activity (Gonda *et al.* 1957). KCN was added to inhibit reoxidation of the reduced TPN through the TPN-cytochrome C reductase-cytochrome oxidase system. Determinations of isocitric dehydrogenase activity employing different levels of isocitrate and TPN in the reaction medium showed that the concentrations of these metabolites as used in all our experiments were sufficient to saturate both mitochondrial and soluble fractions at all levels of enzyme tested.

In the following discussion, the term "larval equivalent" is used to denote the wet weight of larvae from which was obtained the quantity of enzyme preparation tested. Total activity is defined as micromoles of TPN reduced per hour by the total quantity of enzyme used in any test. Specific activity is defined as the micromoles of TPN reduced per hour per 100 mg. wet weight of larvae.

RESULTS.—It is seen from table 1 that when samples from the same preparation of mitochondrial and supernatant fractions were used, the deviation from the mean of six replicates in each determination was very small as compared with the specific activity of TPN-isocitric dehydrogenase and to the effect produced by the addition of DDT. Preparations of mitochondria showed a more uniform response to the addition of DDT than those of the supernatant fraction, although in the absence of DDT, the activity of isocitric dehydrogenase in samples from both cell fractions had low standard errors. This is probably attributable to the fact that mitochondria have a higher content of lipids and therefore may facilitate a more uniform distribution of DDT through the reaction medium. The enzyme activity in the mitochondria of the DDT-R larvae was about 3½ times that for the S larvae and about 1½ times that of the DL-R larvae. TPN-isocitric dehydrogenase activity in the soluble fraction was approximately the same in all cases and was 10 to 40 times the mitochondrial activity, depending upon the strain.

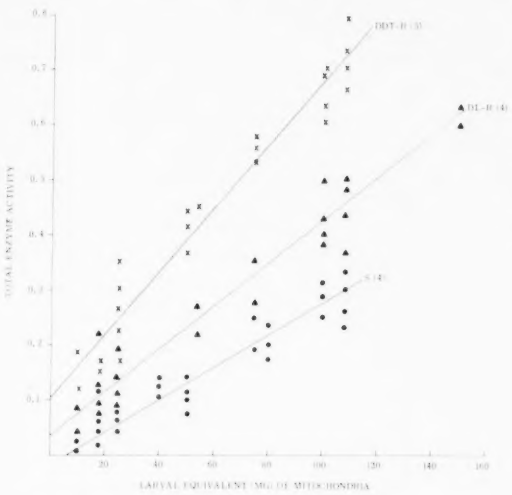

FIG. 1.—Relationship of the concentration of mitochondria to total expressed TPN-isocitric dehydrogenase activity.

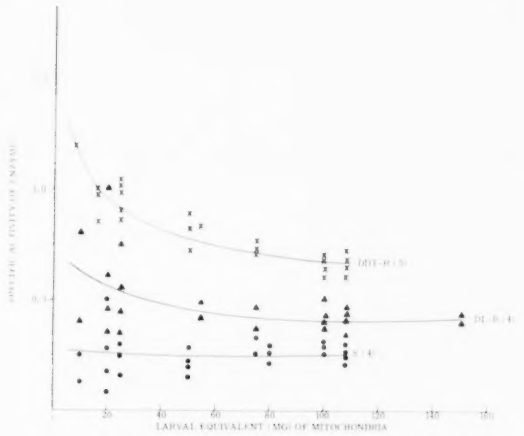

FIG. 2.—Relationship of the specific activity of TPN-isocitric dehydrogenase to the concentration of mitochondria.

Figures 1 through 6 represent composite graphs of several determinations of isocitric dehydrogenase activity conducted on separate preparations of cell fractions at different times during a period of 6 months. The total number of experiments represented in each graph is indicated in parenthesis thereon. It is seen that the total activity of the enzyme increased in all the three strains as the concentration of mitochondria in the reaction medium was increased (fig. 1). On the other hand, the specific activity of the enzyme from the DDT-R strain showed a steep decrease in the range of 10- to 50-mg. equivalents of mitochondria. The specific activity of the DL-R and S strains decreased comparatively slowly in the same range. All three enzyme preparations reached a plateau at concentrations of more than 50-mg. equivalents (fig. 2).

As the concentration of the soluble fraction was increased from 1- to 12-mg. equivalents, the total activity of the enzyme increased in all the strains, but the specific activity had a maximum value at 2.5 mg. of enzyme concentration, on either side of which the values tended to fall (figs. 3 and 4).

When the ratio of DDT to mitochondria in the reaction mixture was increased, there was a steady rise in inhibition of enzyme activity in the S larvae from about 20%

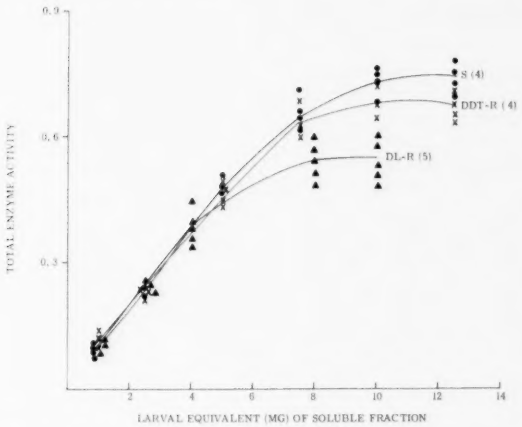

FIG. 3.—Relationship of the concentration of the soluble fraction to total expressed TPN-isocitric dehydrogenase activity.

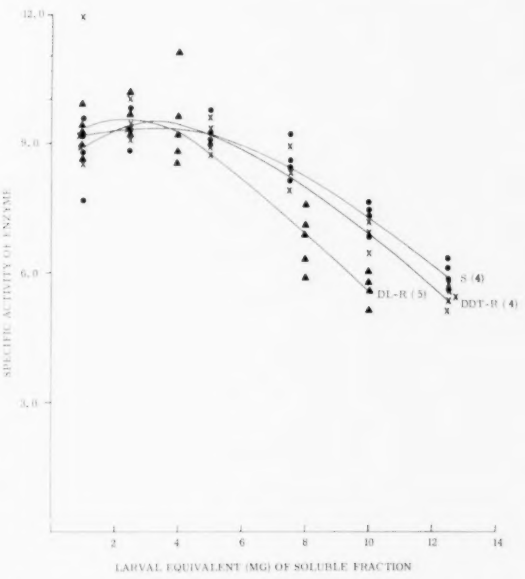

FIG. 4.—Relationship of the specific activity of TPN-isocitric dehydrogenase to the concentration of the soluble fraction.

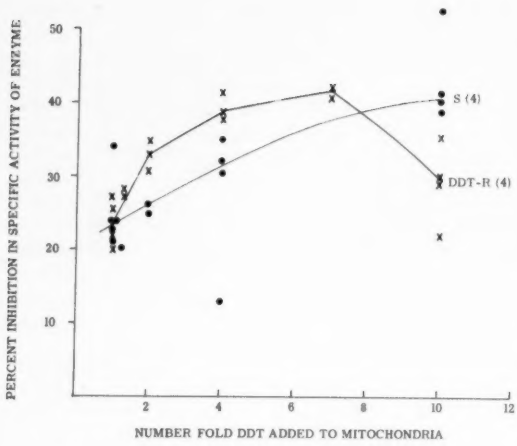

FIG. 5.—Inhibition of TPN-isocitric dehydrogenase activity in mitochondria by DDT.

to 40% as the DDT concentration was increased tenfold. In the DDT-R strain, the same maximum inhibition was reached at a four-fold increase in DDT concentration, after which there was a small decline in inhibition with further additions of DDT (fig. 5).

Two experiments were conducted on the effect of insecticides on the isocitric dehydrogenase activity of the DL-R strain and the mean values are shown in tables 2 and 3. Both DDT and dieldrin added separately to the medium increased the mitochondrial enzyme activity in this strain at all levels of the preparation tested.

As the ratio of DDT to soluble fraction in the reaction mixture was increased, the S strain showed an initial inhibition followed by a steady increase. The soluble isocitric dehydrogenase of the DDT-R strain also increased in proportion to the amount of DDT present (fig. 6). In the DL-R strain, however, both DDT and dieldrin produced an inhibition at all the levels tested when added to the soluble fraction.

It was observed in the course of our experiments with soluble fractions prepared at different times, that although in the majority of preparations from the S and DDT-R strains, there was an increase in enzyme activity on the addition of DDT, in some isolated cases there was a decrease which did not follow a regular pattern. It is suggested that this phenomenon may be caused by the conditions of preparation of the soluble fraction and to the degree to which this fraction became contaminated with mitochondrial constituents during preparation by disruption or leaching.

DISCUSSION.—Enzymes other than TPN-isocitric dehydrogenase shown to be distributed in different cell fractions are aconitase and cytochrome C reductases. Dickman & Speyer (1954) showed that the aconitase activity retained by mitochondria of liver behaved very

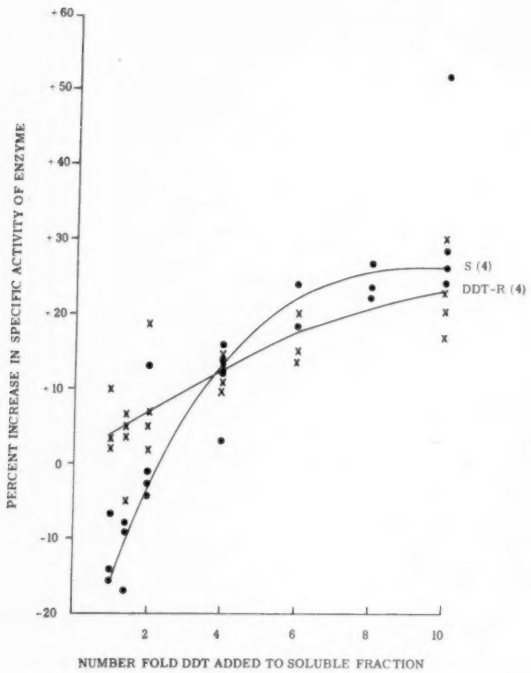

FIG. 6.—Increase of TPN-isocitric dehydrogenase activity in the soluble fraction by DDT.

Table 2.—Effect of DDT[a] and dieldrin[a] on the mitochondrial TPN-isocitric dehydrogenase activity of the DDT-dieldrin resistant strain (DL-R) of larvae of *A. aegypti*.

LARVAL EQUIVALENT OF MITOCHONDRIAL FRACTION	TOTAL ACTIVITY	SPECIFIC ACTIVITY	DIFFERENCE IN SPECIFIC ACTIVITY (%)
25 mg.	0.088	0.354	—
25 mg.+DDT	0.151	0.644	+70.9
25 mg.+dieldrin	0.117	0.469	+32.7
75 mg.	0.338	0.369	—
75 mg.+DDT	0.326	0.435	+17.8
75 mg.+dieldrin	0.365	0.487	+31.7
150 mg.	0.630	0.420	—
150 mg.+DDT	0.731	0.488	+16.1
150 mg.+dieldrin	0.792	0.528	+25.7

[a] 1.1×10^{-4} M.

Table 3.—Effect of DDT[a] and dieldrin[a] on the soluble TPN-isocitric dehydrogenase activity of DDT-dieldrin resistant (DL-R) strain of larvae of *Aedes aegypti*.

LARVAL EQUIVALENT OF THE SOLUBLE FRACTION	TOTAL ACTIVITY	SPECIFIC ACTIVITY	DIFFERENCE IN SPECIFIC ACTIVITY (%)
2.0 mg.	0.183	9.160	—
2.0 mg.+DDT	0.113	5.630	−38.5
2.0 mg.+dieldrin	0.124	6.190	−32.4
4.0 mg.	0.318	7.960	—
4.0 mg.+DDT	0.193	4.825	−39.4
4.0 mg.+dieldrin	0.183	4.580	−42.4

[a] 1.1×10^{-4} M.

differently from the soluble aconitase as to pH optima. They explained this difference in behavior as a physical one involving the mitochondrial membrane. The experiments of deDuve *et al.* (1955) showed that rat liver mitochondrial cytochrome reductases differed from microsomal reductases in that the former were inhibited by Antimycin A while the latter were not. Our studies of TPN-isocitric dehydrogenase in three strains of larvae of *A. aegypti* show that DDT inhibits the mitochondrial enzyme in the S and DDT-R larvae but stimulates an increase in the enzyme from the same subcellular fraction in the DL-R strain. On the other hand, this insecticide increases the TPN-isocitric dehydrogenase activity in the soluble fractions of the first two strains whereas it inhibits it in the third strain. Although further work in our laboratory designed to throw more light on this problem is in progress, it is felt that these preliminary data suggest the possibility that the isocitric dehydrogenases present in different intracellular locations may have different biochemical characteristics, and play roles of greater or lesser importance in the *in vivo* metabolism.

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Effect of Soil Cultivation, Soil Surface and Water on the Persistence of Insecticidal Residues in Soils[1]

E. P. LICHTENSTEIN and K. R. SCHULZ, *Department of Entomology, University of Wisconsin, Madison*

ABSTRACT

Since relatively large amounts of water evaporate yearly from soils, experiments were conducted to obtain more information on the relationship between this water loss and the persistence of residues of insecticides in various soil types. The effect of soil stirring on the persistence of aldrin and DDT residues was also investigated.

It was found that the persistence of aldrin is affected by soil moisture. Water, apparently, causes a displacement of the aldrin from the soil particles. Once this is accomplished, a major part of the insecticide is lost by volatilization. Aldrin is lost from water surfaces, but this loss does not seem to be affected by water evaporation as such.

The persistence of DDT was not affected by the amounts of water evaporated from soils or glass surfaces, or by enlargement of surfaces onto which DDT had been deposited.

Under field conditions, however, daily disking of a loam soil treated with aldrin or DDT at 4 lb./5″ acre caused a reduction of 38% of the aldrin residues and 25% of the DDT residues, during a 3-month period. Frequent soil disking could possibly serve as a method for reducing the residue level of volatile insecticides in soils.

Studies of residues of insecticides in soils during the past 10 years have been concerned, to a large extent, with the determination of the insecticidal residue level as such. However, some work has been done (Bollen *et al.* 1958, Lichtenstein & Schulz 1959a & b, 1960) on the factors responsible for the disappearance of these residues from soils. This line of research seems to be an important one in order to keep residues of insecticides in soils at a reasonable level. As relatively large amounts of water evaporate yearly from soils, it was felt that more information should be obtained on the relationship between this loss of water and the persistence of residues of insecticides in soils. Bowman *et al.* (1959) as well as Weidhaas *et al.* (1960) reported that DDT in a water suspension was lost by "codistillation."

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As soil cultivation may be an additional factor, influencing the loss of insecticides, experiments were conducted on this aspect in the laboratory as well as under field conditions. Glass surfaces, water, and soils were treated with aldrin or DDT and the persistence of the resulting residues as influenced by water evaporation and soil cultivation was investigated.

PROCEDURES.—1) *Laboratory Experiments.*—a) *With Wet Soils.*—A quartz sand and three soil types (Plainfield sand, Carrington silt loam and Muck) were treated with a volatile (aldrin) and a less volatile (p,p'-DDT) insecticide at a rate of 10 p.p.m. by pipetting a measured amount of the chemical onto the soil. After thorough mixing of each soil (Lichtenstein & Schulz 1959a), an aliquot was taken for initial analyses. Equal volumes (148 ml.) of each treated soil (230 g. quartz sand, 167 g. Plainfield sand, 116 g. Carrington silt loam, and 50 g. Muck, ovendry basis) were then placed into either 500 ml. cotton plugged bottles, 500 ml. open bottles, crystallizing dishes or into crystallizing dishes in which the soils were stirred daily with a spoon. Each of the four variations was replicated 2 to 4 times for each soil type investigated. The surface within the bottles onto which the soil was placed was 33.2 square inches and within the crystallizing dishes 74.6 square inches.

All the soil samples were held in an incubator at 37±1° C. They were kept as close as possible to field moisture capacity by adding distilled water either daily or at longer intervals as found necessary. The amounts of water added to each soil were recorded for the duration of the experiments.

The aldrin-treated soils in bottles were analyzed for both aldrin and dieldrin, 56 days after treatment. Those in crystallizing dishes were analyzed 14, 21, 42 and 56 days after treatment. The wet quartz sand, because of its lower insecticide-retention capabilities, was also analyzed 3.5 and 7.0 days after treatment. When DDT was used, the Carrington silt loam was held in both bottles and in crystallizing dishes, whereas the quartz sand, Plainfield sand and Muck were kept in crystallizing dishes only. All soils were analyzed 56 days after treatment for p,p'-DDT and possible breakdown products.

b) *With Dry Quartz Sand.*—To further study the effect of water on the persistence of residues of insecticides in soils, dry quartz sand was treated with either aldrin or DDT. The experiments were set up as described above except that no water was added to the sand during the course of the experiment. Twenty-one days after treatment samples were analyzed for residues of either aldrin or DDT.

c) *With Wet and Dry Glass Surfaces.*—The effect of water evaporation from glass surfaces on the persistence of residues of insecticides was studied. Four ml. of acetone containing 2.4 mg. of either aldrin or DDT were added to various amounts of water within crystallizing dishes. In addition, the same amount of insecticide in acetone was pipetted onto the glass surface of other dishes. Immediately after treatment, all dishes were placed in an incubator at 37±1° C. or 39±3° C. Each dish to which water had been added was left in the incubator until dry. It was then removed together with another dish, to which no water had been added. The containers with aldrin residues were then rinsed quantitatively with hexane and those containing DDT with benzene. The rinsings were adjusted to volume and aliquots were then taken for analyses.

To study the effect of water surfaces on the loss of insecticides from water, 0.2 ml. of ethanol containing 200 μg. of aldrin was pipetted into 20 ml. of water within each of eight 125-ml. Erlenmeyer flasks. The flasks were then plugged with cotton. Four of them were shaken at a speed of 50 cycles per minute for 4 days. The other four flasks were not shaken but left undisturbed in the laboratory (T=74±2° F.) during that time. The water in all flasks was then analyzed for residues of aldrin and dieldrin, using chemical methods and paper chromatography.

The persistence of residues of aldrin and DDT as affected by various surfaces was tested by placing 8 ml. of acetone containing 2.4 mg. of insecticide in 500-ml. glass bottles or crystallizing dishes. After the acetone had been evaporated in a hood one-half of the bottles were plugged with cotton. All containers were then placed in an incubator at 37±1° C. Three days later, the insecticidal residues were rinsed out as described previously and analyzed for residues of either aldrin or DDT.

In another series, the glass surface onto which aldrin was deposited was enlarged by adding either 16 g. of coarse glass beads (3 mm. diameter) or 16 g. of fine glass beads (150 microns diameter) to different crystallizing dishes. As a control, aldrin was added to crystallizing dishes which did not contain glass beads. After 24 hours of incubation at 37±1° C. the aldrin residues were rinsed out quantitatively with hexane and adjusted to volume. Aliquots were then taken for analyses.

2) *Field Experiments.*—In June 1960, two plots (Carrington silt loam, each 30′×40′) were staked out, separated by a 20′ buffer strip. One of the plots was treated with aldrin and the other with DDT each at a rate of 4 lb./5″ acre. The application of insecticide involved a thorough mixing of a measured amount of an emulsifiable concentrate with 20 gallons of water. Each experimental plot was subdivided into five 6′×40′ strips in the south-north direction and into five 8′×30′ strips in the east-west direction. Two-gallon quantities of the diluted emulsion were then spread as uniformly as possible with a sprinkling can over each of the 10 strips. This crosswise treatment provided a more uniform application. After treatment, each plot was rototilled to a depth of 4″ to 5″. From then on one-half of each plot (15′×40′) was disked to approximately 5″ on 5 consecutive days each week for a period of 3 months. The other half was left undisturbed. In October 1960, four months after treatment, the nondisked and disked parts of each plot were sampled and analyzed for residues of insecticides.

3) *Analytical Methods.*—The handling and extraction of the soil samples was done as described by Lichtenstein & Schulz (1959a & b). To the quartz sand samples, which were kept dry during the course of the experiment, water (4 ml./100 g. sand) was added prior to the extraction. This was necessary in order to obtain as complete a recovery of the insecticides as possible. The extraction solvents used were a mixture of redistilled hexane and isopropanol (2:1) for aldrin-treated soils and a mixture

of benzene and isopropanol (2:1) for DDT-treated soils.

The isopropanol was removed from the soil extracts by washing with water. The alcohol free phase was then dried over anhydrous sodium sulfate.

Since extracts of aldrin-treated soils were analyzed for aldrin and dieldrin, cleanup as well as separation of the two insecticides was done by using a column (8″×1″) of magnesium oxide-celite (1:1). After the extract containing aldrin and dieldrin had been added to the column, aldrin was eluted with 100 ml. of redistilled hexane. Dieldrin could then be washed from the column with an additional 500 ml. of the same solvent. Aldrin and dieldrin fractions were analyzed according to the methods of O'Donnell *et al.* (1954, 1955). Known amounts of aldrin added to soils were recovered to an extent of 93% to 97% and known amounts of dieldrin to an extent of 90% to 94%.

Extracts from DDT-treated soils were cleaned up by passing through a column (6″×1″) of aluminum oxide (Merck, Reagent) and analyzed according to the Schechter-Haller Method (1945). Known amounts of p,p′-DDT added to soils were recovered to an extent of 92% to 98%. Since the emulsion used for field treatments contained 84.7% p,p′-DDT, corrections were made accordingly and the final results are expressed for technical material. All results obtained are based on the ovendry weight of the soil under investigation.

In addition to the chemical analyses, ascending paper chromatography as described by Mitchell (1957) was used. R_f values for the different insecticides were established by using extracts from DDT-treated soils, or by using fractions of soil extracts containing either aldrin or dieldrin. They were then compared with R_f values obtained from either p,p′-DDT, o,p′-DDT, DDE, aldrin or dieldrin.

RESULTS AND DISCUSSION.—1) *Laboratory Experiments.*—a) *With Wet Soils.*—When aldrin-treated quartz sand was placed into crystallizing dishes at 37° C. the amount of water evaporated over a period of 21 days from the sand which had been stirred daily was only 1.1 times larger than the amount evaporated from the nonstirred sand. Hence, the major variable in this experiment was the stirring of the soil. However, 3.5 days after treatment the amounts of the residues of insecticides recovered from the stirred sand were 45% of the amount found in the nonstirred sand (table 1). After 7 days incubation the difference was even larger and this figure was only 9.5%. It might be assumed, therefore, that stirring of the sand was responsible for the additional loss of the residues. Twenty-one days after treatment, no residues were recovered from either the nonstirred or the stirred quartz sand. Since this "soil" has a low adsorptive capacity for insecticides and since no dieldrin formation could be noticed, most of the residues of aldrin were probably volatilized.

Table 1.—Residues of insecticides in wet quartz sand, treated with aldrin at 10 p.p.m. in per cent of initial recoveries.[a]

Days After Treatment	Nonstirred Sand		Stirred Sand		
	Aldrin	Dieldrin	Aldrin	Dieldrin	R[b]
3.5	48.1±4.2	0.0	21.2±2.2	0.0	45
7.0	21.3±1.3	0.0	2.0±0.3	0.0	9.5
14.0	0.9	0.0	Trace	0.0	
21.0	0.0	0.0	0.0	0.0	

[a] Temperature = 37 ± 1° C.

[b] R = ratio of residues in $\frac{\text{stirred sand}}{\text{nonstirred sand}} \times 100$.

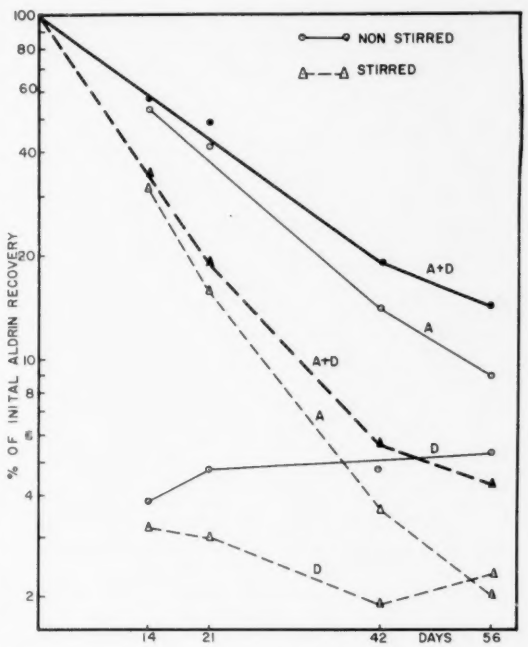

FIG. 1.—Recoveries of aldrin (A) and dieldrin (D) from a Carrington silt loam as affected by soil stirring. Treatment: aldrin 10 p.p.m. Laboratory experiment.

A similar experiment conducted with a Carrington silt loam did not show any appreciable difference in the amounts of water evaporated during 56 days from the nonstirred (1,245 ml.) and the stirred soils (1,400 ml.). However, a marked difference in the amount of residues of insecticides recovered from the nonstirred and stirred loam soil was noticed (fig. 1). This difference increased with time. Fourteen days after soil treatment the total residues found in the stirred soil were still 61% of those recovered from the nonstirred loam. After 56 days, however, this figure amounted to only 30%.

In contrast to the quartz sand, appreciable amounts of dieldrin were found in the loam soil. More dieldrin was recovered from the nonstirred than from the stirred soil. Moreover, the amount of dieldrin in the nonstirred loam increased slightly during the 56 days of incubation, whereas in the stirred soil the peak of dieldrin residues was reached after 14 days of incubation. This latter fact was probably because of the smaller amount of aldrin present in the stirred loam as well as some volatilization of dieldrin from the stirred soil.

Table 2.—Residues of insecticides in quartz sand and three soil types (wet), 56 days after treatment with DDT or aldrin at 10 p.p.m.[a]

		Bottles (33.2 sq. in. surface)			Crystallizing Dishes (74.6 sq. in. surface)			
		(1) Closed	(2) Open		(3) Nonstirred		(4) Stirred	
Soil Type	Organic Matter (%)	T.R.[b]	T.R.	$R\frac{2}{1}$[c]	T.R.	$R\frac{3}{2}$	T.R.	$R\frac{4}{3}$
Quartz sand	0.0							
DDT					82.9± 3.0		73.8± 1.0	89
A+D[d]		1.2± 0.3	0.0		0.0		0.0	
Plainfield sand	0.8							
DDT					97.3± 4.1		93.0± 0.5	96
A+D		61.7± 0.7	38.2± 0.4	63	29.2± 1.2	77	19.3± 0.3	66
Carrington silt loam	4.3							
DDT		65.3± 2.8	73.6± 5.6	113	73.3± 1.0	100	73.1± 3.6	100
A+D		70.2± 1.0	53.9± 1.7	77	14.2± 0.4	26	4.3± 0.2	30
Muck	40.0							
DDT					83.9± 2.7		77.5± 0.5	92
A+D		75.8± 1.8	67.0± 2.1	89	60.9± 4.5	90	36.3± 9.5	60
		Water added during 56 days (ml.)						
		82 ±10	474 ±81		1,169±96		1,234±42	

[a] Temperature, 37± 1° C. (Laboratory experiment).
[b] T.R. = total residues in per cent of initial recoveries.
[c] $R\frac{2}{1}$ = ratio of residues found in $\frac{\text{2) open bottles}}{\text{1) closed bottles}} \times 100$.
[d] A+D = aldrin+dieldrin.

When data from soils in bottles were compared (table 2) with those kept in crystallizing dishes, it seemed that the persistence of the residues of aldrin in all soils investigated was affected by either the different soil surfaces, the amounts of water evaporated from these surfaces, or by both factors. An increase of the soil surface by a factor of 2.3 resulted in a similar increase in the amount of water evaporated from the soil. Fifty-six days after soil treatment, only 30% of the initially applied insecticide was lost from loam soils in closed bottles, whereas a loss of 46% in open bottles and 86% from soils in crystallizing dishes (nonstirred) was registered.

Because of the relatively closed system in which soils were kept in cotton-plugged bottles, various factors might have been responsible for the longer persistence of the residues of insecticides under these conditions.

The persistence of aldrin residues in soils, held in bottles, increased with an increase in the amount of organic matter present in the soils. Most of the aldrin residues were lost in a quartz sand and least in a muck soil. In crystallizing dishes, however, more residues were lost from a loam soil than from a Plainfield sand. It has been reported (Lichtenstein & Schulz 1960) that insecticides persist longer in dry soils than in wet ones. This might have been a factor in the longer persistence of the residues in the Plainfield sand, which dried out faster than the Carrington silt loam. As the soils were watered only once a day, the sandy soil often became dry between waterings.

It should be pointed out, however, that the amounts of water evaporated from the Plainfield sand and the Carrington silt loam were nearly identical. If the insecticide would have been lost from the soil together with the water molecules, the residues found in the two soil types should have been at least similar. But the loss of insecticide was greatest in the loam soil which was wet for 24 hours each day. This might indicate that the insecticide is not taken from the soil with the water as such, but is displaced by the water molecules from the soil particles. Once this factor has been accomplished, other factors are responsible for the loss of the insecticide from the soil.

Data obtained for residues of aldrin and dieldrin show that the smallest amounts of dieldrin were recovered from the aldrin-treated Plainfield sand (table 3). In a Carrington silt loam the epoxidation of aldrin to dieldrin was quite extensive. The absolute amounts of dieldrin produced were largest in those loamy soils which were held in bottles, and smallest in those which were stirred and held in crystallizing dishes. However, the relative amounts of dieldrin (in per cent of the total residue recovered) were largest in the stirred soil because of the higher rate of loss of aldrin.

In the muck soil, aldrin was more persistent than in any other soil type, though evaporation of water or increased soil surface as well as soil stirring affected the disappearance of aldrin residues from the soil. The largest amount of dieldrin (24.7% of the applied aldrin) was found in the nonstirred muck which was held in crystallizing dishes. Stirring of this soil resulted in dieldrin residues amounting to only 60% of those which were recovered from the nonstirred muck.

The persistence of DDT in wet soils, treated with the p,p′-isomer in the laboratory, was not affected by different soil surfaces, by the amounts of water evaporated, nor by soil stirring (table 2). Data on residues obtained from a Carrington silt loam held under the four described conditions were essentially the same. Stirring of the

Table 3.—Residues of aldrin (A) and dieldrin (D) in three soil types (wet), 56 days after treatment with aldrin at 10 p.p.m. in per cent of initial aldrin recovery.[a]

Soil Type		Bottles		Crystallizing Dishes	
		(1) Closed	(2) Open	(3) Nonstirred	(4) Stirred
Plainfield sand	A	58.2±0.7	35.9±0.2	27.7±1.3	18.5±0.2
	D	3.5±0.0	2.3±0.1	1.5±0.1	0.8±0.0
	D.T.[b]	5.7	6.0	5.1	4.2
Carrington silt loam	A	55.0±0.8	37.8±1.2	8.9±0.3	2.0±0.1
	D	15.2±0.5	16.1±0.5	5.3±0.1	2.3±0.1
	D.T.	21.7	30.0	37.3	53.5
Muck	A	65.3±1.4	54.0±1.7	36.2±3.3	21.4±5.3
	D	10.5±0.2	13.0±0.3	24.7±1.1	14.9±4.1
	D.T.	13.9	19.4	40.6	41.1

[a] Temperature 37°±1° C. (Laboratory experiment).
[b] D.T. = dieldrin in per cent of total (aldrin+dieldrin).

quartz sand, Plainfield sand and the muck, did not result in any appreciable differences in DDT residues. Chromatograms obtained from extracts, prepared 56 days after soil treatment, revealed the presence of p,p′-DDT only.

b) *With Dry Quartz Sand.*—Residues of aldrin and DDT were surprisingly persistent in quartz sand, which was dry during the 21 days of incubation (table 4). No DDT was lost from the dry sand in either of the containers used, and all of the applied aldrin was still present when the sand was held in bottles. A slight loss of 10% to 15% of aldrin occurred, however, in the sand held in crystallizing dishes. As reported previously, aldrin disappeared completely within 2 to 3 weeks from a wet sand in dishes and within 4 weeks from open bottles. Therefore, watering of aldrin-treated quartz sand affected the disappearance of the insecticide from the sand.

c) *With Wet and Dry Glass Surfaces.*—When an aldrin acetone solution was added to various amounts of water within crystallizing dishes, it was found (table 5A) that after evaporation of the water nearly all the aldrin residues had disappeared. This loss of aldrin from water was found to be greater than the loss of the chemical from a crystalline film in a dry dish. In this latter case significant residues of aldrin were still recovered 19 to 30 hours after incubation. After 40 ml. of water had been added to each of two dishes, the water in one dish evaporated within 19 hours and in the other one within 21 hours only. Apparently the temperature within this particular incubator was not uniform which resulted in different evaporation times. Though the amounts of water evaporated were identical, the residues found were not. Less aldrin was recovered from the dish in which the water evaporated for a longer period of time. Similar data were obtained, when 65 ml. of water had been added to each of two crystallizing dishes. If the insecticide had been lost with the water molecules, the residues recovered should have been similar.

Shaking of cotton-plugged Erlenmeyer flasks to which 20 ml. of water and 200 μg. of aldrin had been added resulted in a considerable loss of aldrin over a period of 4 days. Only 35±8% of the applied insecticide was recovered from the water after that time, whereas the water which was not shaken still contained 91±5% of the originally added aldrin. No dieldrin formation could be noticed in either case. Since no detectable amounts of

Table 4.—Recoveries of residues of insecticides from a dry quartz sand, 21 days after treatment with aldrin or DDT at 10 p.p.m. in per cent of initial recoveries.[a]

Insecticide	Bottles		Crystallizing Dishes	
	Closed	Open	Nonstirred	Stirred
Aldrin	98.5±1.0	96.6±3.3	86.6±0.15	81.6±4.1
DDT	97.9±0.9	97.6±0.8	99.7±0.7	100 ±0.2

[a] Temperature 37±1° C. (Laboratory experiment).

Table 5.—Recoveries of residues of insecticides from crystallizing dishes, after evaporation of various amounts of water.[a]

Water Evaporated (ml.)	Incubation Time (hours)	Recovered in Per Cent of Applied
	(A) Aldrin	
40	19	0.66
0	19	18.50
40	21	0.22
0	21	9.70
50[b]	30	1.09±0.1
0[b]	30	21.80±0.6
65	24	0.28
0	24	23.90
65	27	0.0
0	27	7.5
	(B) DDT	
25	11	96.5
0	11	95.8
50[b]	24	97.5 ±0.4
0[b]	24	87.1 ±1.5
100	36	98.0
0	36	89.0

[a] At 39±3° C.
[b] At 37±1° C.

Table 6.—Recoveries of residues of insecticides from various glass surfaces in per cent of amount applied.[a]

Insecticide	Incubation Time (days)	Bottles		Crystallizing Dishes
		Closed	Open	
Aldrin	3	48.7±1.76	19.5±0.6	0.04±0.03
DDT	3	100.1±0.25	107.4±5.2	95.5 ±2.2
		From crystallizing dishes		
		1	2	3
		—	16 grams of glass beads	
		—	3 mm. diameter	150 micron diameter
Aldrin	1	16.85±6.85	3.3±0.2	0.6±0.05

[a] Applied 2.4 mg. per container. Temperature 37 ± 1° C.

Table 7.—Residues of DDT, aldrin (A) and dieldrin (D) recovered in a Carrington silt loam, 4 months after treatment with DDT or aldrin at 4 lb./5″ acre.[a]

Insecticide	(1) Non-disked	(2) Disked for 3 Months	$R\frac{2)}{1}$
DDT	74.2	55.9	75
A+D	46.9	29.3	62
A	29.3	19.1	
D	17.6	10.2	

[a] In per cent of amount applied. (Field experiment.)

[b] Ratio of residues found in $\frac{2)\ \text{Disked soil}}{1)\ \text{Nondisked soil}} \times 100$.

water had been lost from any of the Erlenmeyer flasks (shaken or not shaken) the aldrin probably had volatilized at the water-air interface of those water surfaces, which were constantly exposed to air because of the shaking. Therefore, the water surface seems to be one of the contributing factors responsible for the loss of aldrin and not water evaporation *per se*.

DDT was found to be extremely persistent in both water and on glass surfaces (table 5B). Data obtained ranged from 90% to 95% of the amount of DDT which had been added either to various amounts of water or to a dry glass surface.

d) *With Various Glass Surfaces.*—The persistence of residues of aldrin decreased with an increase in glass surface onto which the residues were originally deposited (table 6). Nearly all the aldrin (2.4 mg.) disappeared within 3 days from a glass surface of 74.6 sq. inches (crystallizing dishes), while 79.5% was lost from a surface of 33.2 sq. inches (open bottles). In cotton-plugged bottles the loss amounted to only 51.3%. When the glass surface within the crystallizing dishes was increased by adding glass beads, residues of aldrin disappeared at an even faster rate.

The persistence of DDT on different glass surfaces was not altered.

2) *Field Experiments.*—Under field conditions, however, disking the soil apparently had some effect on the persistence of residues of DDT. While only 26% of the applied chemical was lost during a 4-month period from the nondisked loam plot, a loss of 44% was registered in the soil which had been disked during the first 3 months following soil treatment. The recovery of DDT in the nondisked plot (74.2% of applied) was similar to that obtained under laboratory conditions from the same soil type. Chromatograms prepared from soil extracts showed only the same components of technical DDT as found in the originally applied DDT emulsion (table 7).

The effect of disking on the persistence of residues under field conditions was more pronounced when aldrin had been applied to the soil. A loss in residue of 53% was registered in the nondisked plot and of 70% in the disked one. Yet these losses were still smaller than those registered under laboratory conditions (37° C., 1,400 ml. water evaporated). The average temperature in the field during the time of the experiment was 20° C. and the total rainfall amounted to 19.6″.

The results of all these experiments seem to indicate that disking the soil might be a method for reducing the level of residues of insecticides in soils.

In conclusion it appears that the persistence of aldrin in soils is dependent to a large extent on the presence of water in the soil but not necessarily on the evaporation of water from the soil. Once the aldrin is displaced by water from the soil particles, a major part of the insecticide is lost by volatilization (unpublished data). Stirring the soil (or soil cultivation) and agitation of water result in a loss of aldrin residues due to the repeated exposure of soil or water surfaces to air. The persistence of DDT under laboratory conditions was not affected by soil moisture, nor by soil cultivation.

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Systemic Insecticides as Soil Treatments for Control of the Mimosa Webworm, *Homadaula albizziae*[1]

J. G. Rodriguez,[2] *University of Kentucky, Lexington*

Abstract

The mimosa webworm, *Homadaula albizziae* Clarke, was found in Kentucky by 1950, and is now statewide in distribution. Most of its damage is directed at ornamental plantings of mimosa and locust. Experimental work in control began in 1957. In August, 60 moraine locust trees were sprayed once with dieldrin, malathion or toxaphene emulsion concentrates. Toxaphene was the most effective measured by larval counts made 6 days after application, but by September it was difficult to distinguish treated from untreated trees.

In 1959, the systemic insecticides phorate and demeton in one early season application were tested on locust, using three techniques. Trunk implantation of concentrates, applied directly or encapsulated into holes bored into the trunk gave variable results. Soil injections applied with a hydraulic sprayer using 20 gallons of spray mixture in four to six probes within 3 feet of the tree trunk were effective when the dose was 80 grams of material per 3-inch tree. Foliar applications of demeton and phorate were effective, but one application was not sufficient to keep the trees free of infestation for the whole season.

In 1960, granulated Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio) ethyl phosphorodithioate) and phorate applied to the soil in a 10-foot circle around the tree, worked lightly into the soil and then watered, gave effective control, as did the emulsion concentrate of phorate sprinkled around the tree in a 10-foot circle. Control of three generations of this insect was achieved. An abundant supply of water is probably required for effectiveness. Placing the materials into holes in the ground under the limbspread proved ineffective. Spray treatments alone or combined with granules did not add to the effectiveness. Tests of foliage 5 months after application of Di-Syston and phorate to soil gave strong cholinesterase inhibition.

The mimosa webworm, *Homadaula albizziae* Clarke, was first discovered in the area of Washington, D. C., during the summer of 1940. It has spread rather rapidly since it spread southward and westward to Richmond, Virginia by 1945; Atlanta, Georgia by 1947; Memphis, Tennessee by 1950, and by 1952 it had reached the northern border of Mississippi (Anon. 1954). The last map published on the distribution of this insect (Anon. 1959) shows that the mimosa webworm infests also the southern half of Indiana, southwestern Ohio and southeastern Missouri. Observations by nurserymen and nursery inspectors place its penetration into Kentucky in 1950 and it is now statewide in distribution.

In some areas this insect attacks native honey locust in forest nurseries; however, in Kentucky most of its damage is directed to ornamental plantings of ornamental mimosa (*Albizzia julibrissin* Durazzini) and to moraine locust. Honey locust and moraine locust may be more severely injured than ornamental mimosa since the former make a quick spurt of growth in the spring and little afterward in the season, whereas the ornamental mimosa continues to grow throughout the summer. The injury is caused by a skeletonization of the foliage which turns brown and dies. The larvae web strongly and often the dead foliage remains webbed together until removed by strong winds or heavy rains. The life history of *Homadaula albizziae* has been described by Wester & St. George (1947). In central Kentucky the first signs of injury may be seen by early June and will progress rapidly through the summer months. It has been noted that often trees may escape injury from the first two generations only to be injured severely in late August or in September by the third generation.

Control Experiments with Sprays.—Experimental work to control the mimosa webworm was started in 1957 in central Kentucky when large sections of residential areas with moraine locust plantings were attacked by this insect and DDT which was being used as a spray treatment was not giving satisfactory control. In one test located on a residential street three blocks long in the Meadowthorpe area of Lexington in August 1957, about 60 moraine locust trees, 3 to 5 inches in diameter were employed in an effort to demonstrate an effective way to control the mimosa webworm. Dieldrin, malathion, and toxaphene emulsion concentrates were applied at 0.25, 1.0 and 1.5 pounds per 100 gallons, respectively. DDT had been used on other trees in this area by a commercial firm. Toxaphene was found to be the most effective according to counts of larvae made 6 days after the application. However, there were enough infested trees in the area so that by the third week of September it was difficult to distinguish between the treatments or between treated and untreated trees. It was concluded, therefore, that as many as three treatments would probably have to be made during the season in order to keep ornamental mimosa and honey locust free of injury. Nurserymen and home owners naturally object to such a strenuous spray program.

The use of systemic insecticide offered a possibility. Ripper (1957) has reviewed the field of systemic insecticide efficiency and also of various means of application. Cleveland (1959) considered soil injection a promising means of applying systemic acaricides to fruit trees. Mitchell *et al.* (1960) have considered the insecticide phase as a part of a broader review in the area of absorption and translocation of various compounds in plants.

Methods of Application.—*Tests in 1959.*—A local nursery cooperated in experimental work to study the possibility of obtaining seasonal control of the mimosa

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Table 1.—Tests with demeton and phorate against the mimosa webworm, 1959.

TREATMENT[a]	METHOD OF APPLICATION	DOSE PER TREE (G.)	NO. OF TREES	AVERAGE PER CENT INFESTED 1st Gen. 6/24	2nd Gen. 8/3	3rd Gen. 9/11
Trunk Implantations	*Holes per Tree*					
Demeton, 90% Conc.	4, ¼ in., 1 ml. each	22.7	3	25	40	90
	4, ½ in., 2 ml. each	45.4	3	0	10	25
	4, ½ in., 1 ml. in capsules	22.7	3	25	75	90
Phorate, 47.5% E.C.	3, ½ in., 2 ml. each	17.0	3	25	75	90
	4, ½ in., 1 ml. in capsules	11.4	3	25	25	75
Soil Injections	*Gal. per Tree*					
(3 pts. of emul. conc. per 100 gal.)						
Demeton, 26.2%	10	20.2	4	25	65	90
	15	30.3	4	25	65	85
Phorate, 47.5%	10	40.3	4	15	40	60
	15	60.5	4	25	40	50
	20	80.6	4	0	0	0
Sprays	*Per 100 Gal.*					
(1 qt. per 100 gal.)						
Demeton, 26.2%	0.5 lb.	Run-off	10	0	0	20
Phorate, 47.5%	1.0 lb.	Run-off	10	5	15	50
None (check)			5	25	75	100

[a] Application dates: May 21 for trunk implantation and soil injections, June 4 for sprays.

webworm by using a systemic insecticide in one early season application. One block of moraine locust trees about 3 inches in diameter was available; these trees were randomly selected for the three treatments. The trunk implantations were made by boring three or four ¼- and ½-inch holes at a 45° angle to the main axis of the tree. The holes were about 1½ inches deep and were bored equidistant around the trunk of tree but staggered radially, starting from a distance of 1 foot from the ground up to 2 or 2⅓ feet. Dosing was accomplished by pipetting emulsion or spray concentrates directly into the hole or by inserting the material in a veterinary-type gelatin capsule. The holes were then sealed off with tree dressing compound.

The soil injections were made with a steel-pointed 1-inch pipe having a ¼-inch hole at the tip and a cross-bar handle 3 feet from the tip equipped with a switch valve. This gun was connected directly to a truck-mounted spray rig. Injection to about 1½ feet was easily made with a spray pressure of 50 p.s.i. Four to six probes were made within a distance of 3 feet from the tree base. Applications varied from a total of 10 to 20 gallons of mixture per tree. The mixture contained 3 pints of demeton (2 lb./1 gal.) or 3 pints of phorate (4 lb./1 gal.) per 100 gallons of water. These applications were made on May 21.

Foliar applications were also made. This series was treated on June 4 with commercially available demeton and phorate formulations. The sprays contained 1 quart of either demeton or phorate per 100 gallons of water and were applied at 250 p.s.i. to the point of run-off (table 1).

Results and Discussion 1959.—The first sign of injury in the test block was observed on June 17. At three different times during the season and coincidental with each generation, estimates of infestations were made independently by three workers. These estimates were averaged and are given in table 1. Results of the trunk implantation series were not impressive. There was considerable variation between trees. Also it seemed that the borings would have to be repeated on a yearly basis and it was very doubtful if trees would be able to survive such treatment very long. The implantation treatment with demeton at 45.4 grams per tree showed promise for the control of two generations. It lost effectiveness as the season progressed, however, and by September 11, an estimated average of 25% of the foliage of each tree was webbed. The use of gelatin capsules is probably not advantageous.

The soil injections were easily applied, and the results showed that this series was largely underdosed and that the range of actual material required per 3-inch tree is likely to be more than 80 grams of phorate or demeton (table 1). Also it is indicated that the amount of water going into the treated area is important.

Foliar application of demeton was somewhat more effective than phorate at the concentration used. It was evident, however, that one application was not sufficient to keep the trees free of infestation. No apparent phytotoxicity occurred as a result of any of the treatments used.

Tests in 1960.—The same cooperating nursery provided another block of moraine locust trees. These trees, about 3 inches in diameter, had a limbspread of about 8 feet and were growing on a Kentucky bluegrass type of turf. Twelve treatments, including an untreated check, each replicated five times, one tree per replicate, comprised the experiment which dealt largely with application of granulated Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) and phorate. One series of treatments consisted of broadcasting the granules around each tree in a circle 10 feet in diameter. These applications were worked into the soil lightly with a hand garden tractor.

Table 2.—Tests with systemic insecticides and combinations against the mimosa webworm on moraine locust trees, 1960.

Treatment[a]	Dose per Tree (actual)	Average Infestation (%) 9/8	9/22
Granules, Broadcast			
1. Di-Syston, 5%	4 oz.	0	0
2. Di-Syston, 5%	8 oz.	0	0
3. Phorate, 10%	4 oz.	4	38
4. Phorate, 10%	8 oz.	0	1
Granules, in 6 Holes (1½"×12")			
5. Di-Syston, 10%	4 oz.	32	72
6. Phorate, 10%	4 oz.	34	88
Sprinkled Emul. Conc.			
7. Phorate, 47.5%	4 oz.	3	8
8. Phorate, 47.5%	8 oz.	3	6
Sprays—Granules			
9. Toxaphene, 60% E.C. *plus* demeton, 26.2% E.C.	2 lb. / 0.5 lb. per 100 gal.	13	39
10. Phorate, 10% granules broadcast *plus* toxaphene, 60% E.C. spray *plus* phorate, 47.5% E.C. spray	4 oz. / 2 lb. / 1 lb. per 100 gal.	1	1
11. Di-Syston, 5% Gran. Broadcast *plus* toxaphene, 60% E.C. spray *plus* demeton, 26.2% E.C. spray	4 oz. / 2 lb. / 0.5 lb. per 100 gal.	3	2
12. Check, untreated		60	97

[a] Treatments to 5 one-tree replicates: 1 to 8 applied May 19; 9 to 11 applied June 29.

In another series, granules were poured into six spaced holes within a radius of 4 to 5 feet from the trunk of each tree, each hole being 1½ inches in diameter and 12 inches deep. In another series of treatments, emulsion concentrates were mixed with water in a sprinkling can and sprinkled around the tree in a circular area 10 feet in diameter. These aforementioned applications of granules, treatments 1 to 8, were made on May 19 after which the treated area around each tree was watered with a garden hose until soft.

The next series of treatments were to have been made when the first signs of injury appeared on the experimental plots but when no sign of infestation had developed by June 29, it was decided to proceed with the treatments anyway. These treatments consisted of sprays only or sprays combined with granules (table 2).

Results and Discussion 1960.—The first generation did not appear in the test block. On July 27 some three trees showed traces of injury and the first second-generation larvae were seen then. By mid-August the untreated check trees were badly injured, and by September 8, an average of 60% of the foliage was webbed-over (table 2). The estimates of foliage infested represent the independent estimates of three individuals. The final ratings, made on September 22, showed the effectiveness of Di-Syston and phorate granules when they were broadcast in a circle 10 feet in diameter and worked into the soil lightly and then watered. Also impressive was the sprinkling can method of application. The granules were not effective when placed in holes which were then plugged shut. The combination of granules and systemic sprays were effective but this level of control was not any greater than that obtained by the granules alone. The nonsystemic insecticide toxaphene was applied to provide immediate control of larvae while the systemic materials took effect. This plan was inoperative because the first generation of mimosa webworms failed to develop and the application of toxaphene was not needed. No apparent phytotoxicity occurred as a result of any of the treatments.

DISCUSSION AND CONCLUSIONS.—The results show that while trunk implantation offers some possibility as a means of introducing systemic insecticide into moraine locust trees, it is apparently translocated unevenly to the serial portions of the tree. This was evident in the variation in control obtained which confirms the work done by Bowman & Casida (1958) on cacao trees in which they studied distribution of a phosphorus based systemic compound (Chipman R-6200) labeled with P^{32}. There are other definite objections to pursuing a method where holes are drilled into tree trunks. Holes weaken the tree and also may serve as a point of entry for disease organisms. Examination of these trees, treated the previous year with demeton and phorate, showed that these materials were not effective in the second season. Repeated trunk implantation, therefore, would be questionable. Placing the material in holes in the soil under the limb-spread was another method which proved to be inadequate. The reason is undoubtedly one of uneven distribution and limited uptake of the material. The fact that all treatments with granules that were broadcast or with the emulsion concentrate mixtures that were sprinkled-on were immediately provided with a liberal soaking with water should be noted. Also it is important to note the unusually high rainfall that occurred during the 7-day period following the application on May 19, 1960. During this period, more than 3 inches of rain fell. Moreover, this was followed by more than 12 inches of precipitation in the next 4 weeks. The effectiveness of these soil treatments was probably caused in no small measure by this large amount of soil moisture which transported the Di-Syston and phorate to the root zone. Foliage collected in late September 1960 from trees receiving the treatments of broadcast granules (treatments 1–4, table 2) was tested for cholinesterase inhibition. These tests showed that residues of insecticides remaining on the foliage from the application of 4 and 8 ounces of Di-Syston or phorate made on May 19 were still very marked.

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Laboratory Tests of Some Insecticides Against Adults of the Apple Maggot, *Rhagoletis pomonella*[1]

C. W. Maxwell, *Plant Pathology and Entomology Section, Research Station, Canada Department of Agriculture, Fredericton, N. B.*

Abstract

In laboratory tests against the adults of the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Trypetidae), seven insecticides were rated, in descending order, as follows: Diazinon®, Guthion®, malathion, Sevin®, DDT, calcium arsenate, and lead arsenate. Calcium arsenate used with Bordeaux mixture was as effective as Sevin, and the addition of nicotine sulphate increased the effectiveness to that of malathion. A mixture of lead arsenate and DDT was more effective than either used alone. Some loss of toxicity occurred when Diazinon or Sevin was combined with the fungicides glyodin, captan, dichlone, and Bordeaux mixture. Lead arsenate increased slightly in effectiveness when combined with captan but not with glyodin or dichlone.

Diazinon is *O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thiophosphate

Guthion is *O,O*-dimethyl S-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate

Sevin = 1-naphthl *N*-methylcarbamate

The apple maggot, *Rhagoletis pomonella* (Walsh), has increased in numbers in New Brunswick since the so-called mild organic fungicides came into use. Satisfactory control had previously been obtained with calcium arsenate at 3 pounds per 100 gallons in combination with Bordeaux mixture or lime-sulphur-iron sulphate mixture. Lead arsenate had also been effective at 3 pounds per 100 gallons alone or in combination with lime sulphur.

This is a report on laboratory tests of combinations of insecticides and fungicides recommended in the past and at present and of some of the newer insecticides used alone and in combination with some of the newer organic fungicides.

Methods.—Pupae from maggot-infested apples were stored at approximately 32° F. for 10 weeks, and then held at a controlled temperature of 75° F. and an approximate relative humidity of 95%. The adults began to emerge a month later. Flies 1 day old were used in the tests. Generally, enough flies emerged daily for one test, but during the peak of emergence there were enough for two or three tests. Since the susceptibility of the flies may not have been constant during the emergence period, all of the tests were repeated twice, the order of treatments being the same in the three replications. The mortalities that occurred on the corresponding days of exposure in the three replications of each test were summed, as were the numbers of flies that survived the exposure period.

One exposure cage was used for each test. The cage was 1 foot deep, 1 foot wide, and 2 feet high. The bottom and sides were made of plywood; the front was a sliding glass panel; the top and back were covered with fibre-glass cloth. Each cage held 12 symmetrical, full-sized McIntosh Red apples impaled, at their blossom ends, on nails driven up through removable strips of wood. The tops of the apples were about 2 inches from the top of the cage.

The apples were sprayed to dripping with freshly prepared formulation, a laboratory-made glass atomizer being used at a pressure of 20 pounds per square inch. The atomizer was also used in applying lead and calcium arsenate each as a dust without diluent in amounts sufficient to cover the apples in a fine even coating. Twenty-four hours after treatment the apples were placed in a cage containing the flies that had emerged during the previous 24 hours. The cage was then transferred for 21 days to an artificially illuminated room and held at a temperature of 75° F. and a relative humidity of 90% to 95%. The flies were fed a 10% honey solution to which was added a small quantity of brewers' yeast. The solution was applied to three absorbent cotton pads placed on the cloth-mesh tops of the cages. Each day the pads were dampened sufficiently with the solution to fill the interstices of the mesh without dripping. Cages containing flies on untreated apples were maintained during the tests, methods of calculations of mortalities and toxicity ratings being the same as in treatment tests. The mortality was recorded daily for the 21 days of exposure.

The effectiveness of the treatments was determined as a toxicity rating calculated from the following equation:

$$\text{toxicity rating} = 100 \times \left(1 - \frac{\text{actual survival time in fly-days}}{\text{total survival time in fly-days}}\right).$$

The actual survival time in fly-days was the total number of days all flies remained alive and was calculated by summing the number of days each fly remained alive. The total survival time in fly-days was the number of flies used in the test multiplied by 21, the number of days of the exposure period.

The 21-day exposure period represented the maximum amount of time allowed between applications for control of this pest in the orchard. Because of this long exposure period and the slow toxic effect of the arsenates, toxicity ratings more accurately indicated the effectiveness of the spray mixtures than the per cent mortalities. Materials having slow toxic effects increased the number of survival days and lowered the toxic ratings. The peak day of mortality, the day on which most flies died, also indicated the rapidity of the toxic action.

This method of toxicity rating is concerned only with the time required to bring about the deaths of the individuals. The possible effects of the treatments on mating or oviposition, as affecting control of the insect, cannot be measured by this technique of rating.

The materials tested are shown in table 1.

Results and Discussion.—The toxicity ratings were lower for lead arsenate than for the other insecticides. At

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5 pounds of lead arsenate per 100 gallons the rating was not greater than at 4 pounds. Three pounds of lead arsenate per 100 gallons, in combination with captan, was evidently about as effective as 4 or 5 pounds used alone. This result may be explained by some tests previously conducted in the laboratory; the water-soluble arsenic content of lead arsenate in combination with captan and calcium arsenate used alone was about eight times as high as lead arsenate used in combination with glyodin, dichone, or Triton B 1956. The toxicity rating of lead arsenate at 3 pounds per 100 gallons in combination with Triton B 1956 was equal to that of the lead arsenate and captan combination. The ratings did not increase with the addition to lead arsenate of glyodin or dichlone, or of Staley's bait or soy hydrolysate. These insect lures were added to determine if they had any effect on the action of the lead arsenate. Lead arsenate applied as a dust was more toxic than as a spray. It approached lead arsenate plus captan in toxic rating but was slower acting than any of the other arsenical combinations. The peak of mortality for lead arsenate occurred on the 13th day of exposure.

Calcium arsenate dust was superior to lead arsenate dust. It caused 100% mortality, and its toxicity rating was about double that of any of the lead arsenate preparations. The peak mortality of flies occurred on the 12th day. Unfortunately, calcium arsenate cannot be used alone, in either dust or spray form, because of phytotoxic effects. In combination with Bordeaux mixture it gave 100% mortality, but the toxicity rating decreased by 10 points, and the peak day of mortality moved from the 12th to the 13th day. Addition of Goulac (lignin pitch), commonly used as a sticker-spreader, reduced the effectiveness of calcium arsenate. The most effective arsenical combination was the calcium arsenate and Bordeaux mixture plus nicotine sulfate.

Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thiophosphate) and Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate) were more toxic to apple maggot adults than any of the other insecticides that were investigated (table 1). The toxicity rating for Diazinon was 100 and for Guthion somewhat less. When Diazinon was combined with the various fungicides its effectiveness decreased, but the addition of fungicides did not change the toxicity rating of Guthion. Sevin® (1-naphthyl *N*-methylcarbamate) did not cause 100% mortality, but its toxicity rating increased with concentration and its effectiveness was evidently reduced when it was mixed with fungicides, particularly glyodin.

The toxicity rating of DDT at 1 pound per 100 gallons was 39. The toxicity rating of malathion was higher than that of DDT or DDT-lead arsenate and the peak day of mortality was reached more quickly than with DDT or with DDT-lead arsenate.

Natural mortality was recorded continuously throughout the tests from three successively established cages and amounted to only three in terms of toxicity rating. The adopted technique was found the most satisfactory in the reduction or elimination of natural mortality and was arrived at only after many preliminary investigations in temperatures, humidities, type size of cages and feeding solutions.

Table 1.—Toxicities of various insecticides alone and in combination with various fungicides[a] against adults of *Rhagoletis pomonella* during 21 days' exposure to spray residues on McIntosh Red apples.

Technical Material per 100 Imperial Gallons (lb.)	Number of Flies[b]	Mortality (%) Range of 3 Replications	Toxicity Rating[c]	Peak Day of Mortality
Lead arsenate				
3	112	52–72	20	15
4	132	79–91	33	14
5	157	76–83	30	14
Lead arsenate				
3+captan	86	74–75	29	14
3+glyodin	143	55–75	18	16
3+dichlone	80	57–73	18	16
3+Triton B 1956	140	78–90	30	15
3+Staley's bait	95	54–83	23	16
3+soy hydrolysate	130	75–79	22	16
Lead arsenate (dust)	92	95–97	27	17
Calcium arsenate (dust)	106	100	51	12
Calcium arsenate				
3+Bordeaux	78	100	41	13
3+Bordeaux+Goulac	131	87–91	37	14
2+Bordeaux+nicotine	96	100	56	10
Diazinon, 25% W.P.				
4	453	100	100	1
2	121	100	100	1
1	121	100	100	1
Diazinon				
1+glyodin	172	100	86	4
1+captan	218	100	98	1
1+dichlone	214	100	98	1
1+Bordeaux	114	100	90	3
Sevin, 50% W.P.				
1	99	74– 77	42	10
1.5	123	96– 98	77	5
2	157	96– 98	93	5
1.5+captan	303	81– 92	47	10
1.5+glyodin	320	55– 88	33	13
1.5+dichlone	133	90–100	71	6
Guthion, 25% W.P.				
1	43	100	89	3
1.5	92	100	86	4
2	46	100	98	1
1.5+captan	150	100	90	3
1.5+glyodin	144	100	90	3
1.5+dichlone	76	100	98	1
DDT, 50% W.P., 1	115	83–100	39	12
DDT, 1+lead arsenate, 2	157	93–100	47	11
Malathion, 50% W.P., 1	186	88– 90	54	9
Untreated	121	0.7–9	3	15

[a] Captan (*N*-(trichloromethylthio) 4-cyclohexene-1,2-dicarboximide) (Standard Oil Development Co.), was used at 2 pounds per 100 gallons; glyodin (2-heptadecyl-2-imidazoline acetate) (Carbide & Carbon Chem. Co.), at 1 quart; dichlone (2,3-dichloro-1,4-naphthoquinone) (U. S. Rubber Co.), at 0.5 pound; Bordeaux as an 8-25-100 mixture; Triton B 1956 (Rohm & Haas Co., West Hill, Ont.) and Goulac (S. B. Pennick & Co., New York) at 4 ounces; Staley's bait No. 7 (A. E. Staley Manufacturing Co., Decatur, Ill.) and soy hydrolysate at 1 pint; nicotine sulfate at 0.75 pint; Diazinon (J. R. Geigy A. G.); Guthion (Chemagro Corporation); and Sevin (Union Carbide Chemicals Corporation).

[b] Total of three replicates.

[c] Calculated from the formula given in text.

These results confirm observations and tests in orchards. Thus, calcium arsenate in Bordeaux mixture, which provided satisfactory control of the apple maggot before the mild organic fungicides were introduced, had the highest toxicity rating of the arsenates tested. Adding nicotine sulfate to this combination further increased its toxicity rating. Nicotine sulfate was commonly used in the calcium arsenate-Bordeaux combination for control of larvae of the summer generation of the eye-spotted bud moth, *Spilonota ocellana* (D. & S.), a serious pest in many New Brunswick orchards for a number of years; the apple maggot was adequately controlled in such treated orchards. The low toxicity ratings shown for lead arsenate-organic fungicide combinations agree with the

unsatisfactory control reported by New Brunswick apple growers. Orchard trials conducted by the author indicated no benefit in the use of Staley's bait or soy hydrolysate in lead arsenate sprays; similar results were shown in the laboratory tests. Diazinon and Guthion were as effective in field trials conducted by the author as in the laboratory tests, except that in one orchard Diazinon gave little or no control. The treatment now recommended, lead arsenate in combination with DDT, was very effective on late varieties of apples in one year's testing in one orchard; in laboratory tests the toxicity rating was 47 for this combination.

Stable Fly Tolerance to Residues of DDT, Dieldrin, Malathion and Diazinon[1]

LAWRENCE JOHNSTON[2] and THEODORE E. BLAKESLEE[3,4]

ABSTRACT

In tests conducted to determine the insecticide tolerance of a strain of stable flies (*Stomoxys calcitrans* (L.)) from the U. S. Army Chemical Center, Maryland, females evinced an LC-50 of 1.3 mg. per square foot in the case of DDT, 0.8 mg. per square foot for malathion and 0.23 mg. per square foot for Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) 24 hours after a 15-minute exposure in treated pint Mason jars. A significantly lower tolerance to DDT was demonstrated by females held for 24 hours at 70° F. than was demonstrated by females held at 90° F. after a 15-minute exposure. This difference was not significant when similar tests were conducted with malathion. The World Health Organization test procedures for determining the susceptibility or resistance of adult mosquitoes to insecticides was highly satisfactory for testing adult stable flies. Groups of both sexes were tested with this procedure. The slightly higher DDT tolerance evinced by the males was not significant. Females demonstrated a significantly higher tolerance to dieldrin than the males.

This study was conducted with adult stable flies (*Stomoxys calcitrans* (L.)) to determine a) their tolerance to DDT, malathion, dieldrin, and Diazinon (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), b) whether temperature affected their response to insecticides, and c) the feasibility of using the World Health Organization adult mosquito insecticide resistance kit to determine their insecticide tolerance.

Insecticide tolerance levels have been determined for a number of medically important arthropods. Data concerning the insecticide tolerance of house flies, German and American cockroaches, and 14 species of mosquitoes are listed in Technical Information Memorandum No. 3. The effect of temperature on insects exposed to insecticides has been studied among several medically important species. Shepard (1958) has reviewed this subject extensively.

MATERIALS AND METHODS.—The study was made with a laboratory strain that was established at the U. S. Army Environmental Health Laboratory, U. S. Army Chemical Center, Maryland. The colony was started with adults collected from animal pens at the Army Chemical Center's Chemical Warfare Laboratory in October 1957. The rearing method was essentially the same as that described by McGregor & Dreiss (1955).

The colony was housed in an insectary which was maintained at 80° F. and 75% relative humidity. Adults were confined in screened rearing cages approximately 30 inches long, 18 inches wide, and 18 inches high. The rearing cages were housed on a shelf under a table. A sheet of black cloth was secured to the edge of the table top and draped over the front of the cages to exclude light. A 3-inch strip was removed from the back portion of the table top so that a section of each rearing cage was exposed to light. The strip was covered with a ½-inch-mesh wire screen. Adults were fed citrated bovine blood which was dispensed in 8-mm. glass tubes approximately 6 inches long. One end of each tube was fire-polished to reduce the rate of flow. The other end was provided with a rubber bulb from an eye dropper to facilitate refilling the tube. After each tube was filled, it was inserted through the ½-inch-mesh screen with its open end resting on the cage screen top. Tubes were filled with blood twice daily. Light to attract flies to the blood source was provided by fluorescent lamps. These were suspended above the blood tubes and burned constantly.

At first, eggs were collected on black cloth which enclosed a cotton wad dampened with an ammonium carbonate solution. This method was changed to collecting the eggs on petri dishes of CSMA (Chemical Specialties Manufacturers Association) fly larval medium which had been mixed with water several days previously. Eggs were transferred to prepared medium in 2-gallon glass animal jars. Medium was prepared by mixing 400 grams of CSMA fly larval medium with 850 ml. of tap water. When pupation was completed, the top 2 inches of the medium was transferred to a shallow pan where it was dried and the pupae separated from the medium by an electric fan.

Only engorged flies were used in the tests. The following procedure was used to ensure that sufficient engorged

[1] This material has been reviewed by the Office of The Surgeon General, Department of the Army, but does not imply any indorsement of the opinions advanced or any recommendation of such products as may be named. Accepted for publication November 22, 1960.

[2] Department of Entomology, University of Maryland, College Park.

[3] Sixth US Army Medical Laboratory, Fort Baker, California.

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Table 1.—Per cent mortality of female stable flies 24 hours after a 15-minute exposure.

Insecticide	Number Used at Each Dosage	Dosage (mg. per square foot)									
		0.1	0.20	0.25	0.40	0.50	0.80	1.0	1.6	2.5	5.0
DDT	58–63	5	—	8	—	23	—	39	—	69	95
Malathion	89–101	23	—	28	—	38	—	73	—	86	87
Diazinon	130	17	50	—	61	—	96	—	100	—	—

adults were available at the time of testing. The blood source was withdrawn from the flies scheduled for testing by 3:30 p.m. on the afternoon before the test day. The test flies were again provided blood at 7:00 a.m. on the test day. By 9:00 a.m. there were sufficient engorged flies for testing. Flies 4 to 7 days old were used in all tests. They were anesthetized with carbon dioxide and segregated as to sex.

(1) *Tolerance of Stable Flies to Residual Deposits of DDT, Malathion and Diazinon.*—The Armed Services House Fly Test Procedure (anonymous 1959) was used to determine the tolerance of female *Stomoxys calcitrans* to residual deposits of DDT, malathion and Diazinon. Females were confined for 15 minutes in pint Mason jars whose interiors had been treated with known concentrations of insecticide in acetone. The mouth of each jar was covered with a card treated with repellent. At the end of the exposure period, the flies were transferred to insecticide-free Mason jars and held for 24 hours in a constant temperature cabinet where the temperature was maintained at 80° F. and the relative humidity at 75%. Mortality was recorded at the end of the 24-hour holding period. Mason jars were treated approximately 2 hours prior to the exposure of the test flies. Ten females were confined in each jar.

(2) *Effect of Temperature on Mortality of Stable Flies Treated with Insecticide.*—Tests were conducted to determine whether the temperature at which stable flies were held during the 24-hour holding period subsequent to insecticide exposure affected test results. Ten test groups of females were tested with DDT and malathion using the Armed Services House Fly Test Procedure (anonymous 1959). Each test group consisted of two test series. Both test series were exposed to the following insecticide doses at 80° F.: 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 milligrams of insecticide per square foot. Ten females were exposed to each insecticide dose in each test series. At the end of the exposure period, one series was held for 24 hours in a constant temperature-humidity cabinet (Aminco Aire) at 70° F. and at 75% relative humidity; the other series was held in a small room at 90° F. and at 75% relative humidity. Ten of these groups were exposed to DDT and 10 to malathion.

(3) *World Health Organization Test Procedure for Determining the Tolerance of Adult Mosquitoes to Insecticides.*—Stable flies were tested with the World Health Organization method for determining insecticide tolerance of adult mosquitoes (Mathis *et al.* 1959). Groups of engorged adults of the same sex were confined in plastic tubes (10 per tube) lined with insecticide-treated paper. At the end of a 30-minute exposure period, the adults were transferred to plastic tubes lined with untreated paper and held for 24 hours. Mortality was recorded at the end of the 24-hour holding period. Groups of each sex were tested to determine whether there was a difference in insecticide tolerance between the sexes. These tests were conducted with DDT and dieldrin.

Results and Discussion.—(1) *Stable Fly Tolerance to Residues of DDT, Malathion and Diazinon as Determined by the Armed Services House Fly Test Procedure.*—Test results are contained in table 1. The LC-50's and LC-90's of engorged females 24 hours after a 15-minute exposure were calculated by plotting a concentration-mortality regression line on log probit paper. The LC-50 was determined to be 1.3 mg. per square foot for DDT, 0.8 mg. per square foot for malathion, and 0.23 mg. per square foot for Diazinon. The LC-90 was 5.4 mg. per square foot for DDT, 2.6 mg. per square foot for malathion and 0.98 mg. per square foot for Diazinon.

(2) *Temperature Effect on the Mortality of Insecticide-Treated Stable Flies.*—Test results are presented in table 2. The LC-50 for flies exposed to DDT residues was calculated to 0.3 mg. per square foot for test groups held at 70° F. and 1.24 mg. per square foot for test groups held at 90° F. This difference was determined to be significant to

Table 2.—Per cent mortality in female stable flies held at 70° F. and 90° F. after exposure to DDT and malathion residues.

Insecticide	Temperature (° F.)	Concentration of Insecticide Residue (mg. per square foot)					
		0.1	0.25	0.5	1.0	2.5	5.0
DDT	70	25[a]	41	67	87	99	100
	90	10	17	21	37	73	93
Malathion	70	28	50	65[a]	84	96	95
	90	54	60	71	80	91	94

[a] 99 test flies; 100 used in all other tests.

Table 3.—Twenty-four-hour mortalities (%) of adult stable flies after 30-minute exposures to DDT and Dieldrin-Risella oil impregnated papers.

DDT			Dieldrin		
Insecticide Concentration (%)	Male	Female	Insecticide Concentration (%)	Male	Female
0.25	4	8	0.05	10	6
0.5	6	12	0.1	28	10
1.0	16	28	0.2	54	48
2.0	48	56	0.4	80	50
4.0	94	98	0.8	78	54
			1.6	84	94

the 1% level. The difference in tolerance obtained at the two temperatures with malathion was not found to be significant.

(3) *World Health Organization Test Procedure for Determining the Tolerance of Adult Mosquitoes to Insecticides.*—Results of these tests are given in table 3. In the case of DDT, the LC-50 was 1.3% in males and 1.8% in females. In the case of dieldrin, the LC-50 was 0.20% in males and 0.52% in females. Treatment of the data by the analysis of variance indicated that this difference in insecticide tolerance evinced by the sexes is not significant in the case of DDT, but is significant where dieldrin is concerned. The World Health Organization Procedure for determining the insecticide tolerance of adult mosquitoes was found to be highly satisfactory in determining insecticide tolerance in *Stomoxys calcitrans* adults. The adults frequently defecated, quickly soiling the test papers, making it necessary to use fresh papers with each test.

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Life History and Biological Control of *Caligo eurilochus*, A Pest of Banana[1]

FEDERICO MALO, *Department of Research, Compania Bananera del Ecuador, Guayaquil*[2] and EDWIN R. WILLIS,[3] *Central Research Laboratories, United Fruit Company, Norwood, Mass.*

ABSTRACT

The larvae of this Ecuadorean insect (Lepidoptera: Nymphalidae) feed extensively on banana leaves. There are five larval instars during a two-month period; pupal development takes 18 days, and the adult lives about 55 days. The various stages are described and, in part, depicted in photographs. Details of the insect's behavior and its relation to the host plant are given.

Eight parasites attack the immature stages: an egg parasite, *Xenufens* sp. near *ruskini* Girault (Trichogrammatidae); a larval parasite, *Apanteles opsiphanes* Schrottky (Braconidae); the larval and pupal parasites, *Sarcophaga lambens* Wied; *Sarcophaga* sp. (Sarcophagidae), *Achaetoneura* sp., *Winthemia* sp. (Tachinidae), and *Megaselia* sp. (Phoridae); and a pupal parasite, *Megaselia* sp. An undetermined hemipteron preys on both larvae and pupae. Details of these associations together with data on the control effected by the parasitic flies are given.

In the coastal region of Ecuador a large brown caterpillar ("la vaquita negra") attacks the leaves of the commercial banana. In the absence of effective biological control (described below) this insect could easily become a serious banana pest. Adult specimens of this insect were determined to be *Caligo eurilochus* (Cramer 1775) (Lepidoptera: Nymphalidae) by W. D. Field, United States National Museum.

In our collaboration, Malo made the observations on *C. eurilochus*, its parasites and predator, and control, in the field and laboratory at Tenguel, Ecuador; Willis confirmed and extended some of the observations on *C. eurilochus* in the laboratory, took the photographs, processed the data, and prepared the final manuscript for publication.

DESCRIPTIONS.—*Adult.*—The wing spread of *C. eurilochus* is about 5 to 6 inches, the female being slightly larger than the male (figs. 1, 2). Dorsal wing surfaces of both sexes are characterized by apical bands of dark brown scales which are gradually replaced basally by dark grayish-blue scales. Interspersed among the flat blue scales on the base of the wings are long, gray, hairlike scales that resemble brushed wool. Light brownish-buff scales occur along the anal margins of the hind wings; these are more distinct in the male than in the female. There are androconia on the male hind wings at the junction of the blue and buff scales opposite the posterior part of the abdomen.

Ventrally, the wings of both sexes are mottled with a wavy pattern of dark brown and white or tan scales (figs. 3, 4). Each of the hind wings bears three eye spots; the posterior and largest spots are about three-quarters of an inch across. When spread specimens are inverted, the ventral surface suggests the common name, owl butterfly.

The body, which is $1\frac{1}{4}$ to $1\frac{3}{4}$ inches long, is covered with grayish-brown scales with a faint dusting of blue. The male abdomen, which is more slender than that of the female, bears conspicuous scent glands on each side (fig. 2) opposite the androconia on the wings; these glands are covered with reddish or yellowish scales. Similar abdominal scent glands have been described and figured by Barth (1953) for *Caligo arisbe* Hübner.

Egg.—Newly laid eggs are pearly white, 1.7 to 2.2 mm. in diameter and length, subspherical, and meridionally ribbed (fig. 5A). The ventral pole is flattened where the egg is attached to the leaf (fig. 5B). Between the 26 to 27 ribs the chorion is depressed and finely reticulated.

First-Instar Larva.—The newly hatched larva is about 8 mm. long, including the bifid anal process which is about 2 mm. long. The subspherical, light-brown head, which is covered with long black setae, is $1\frac{1}{2}$ to 2 times the width of the body. The body is creamy white with narrow, reddish-brown bands extending laterally from the neck to the posterior end of the abdomen. Each arm of the forked anal process is pale brown with a dark tip; each arm

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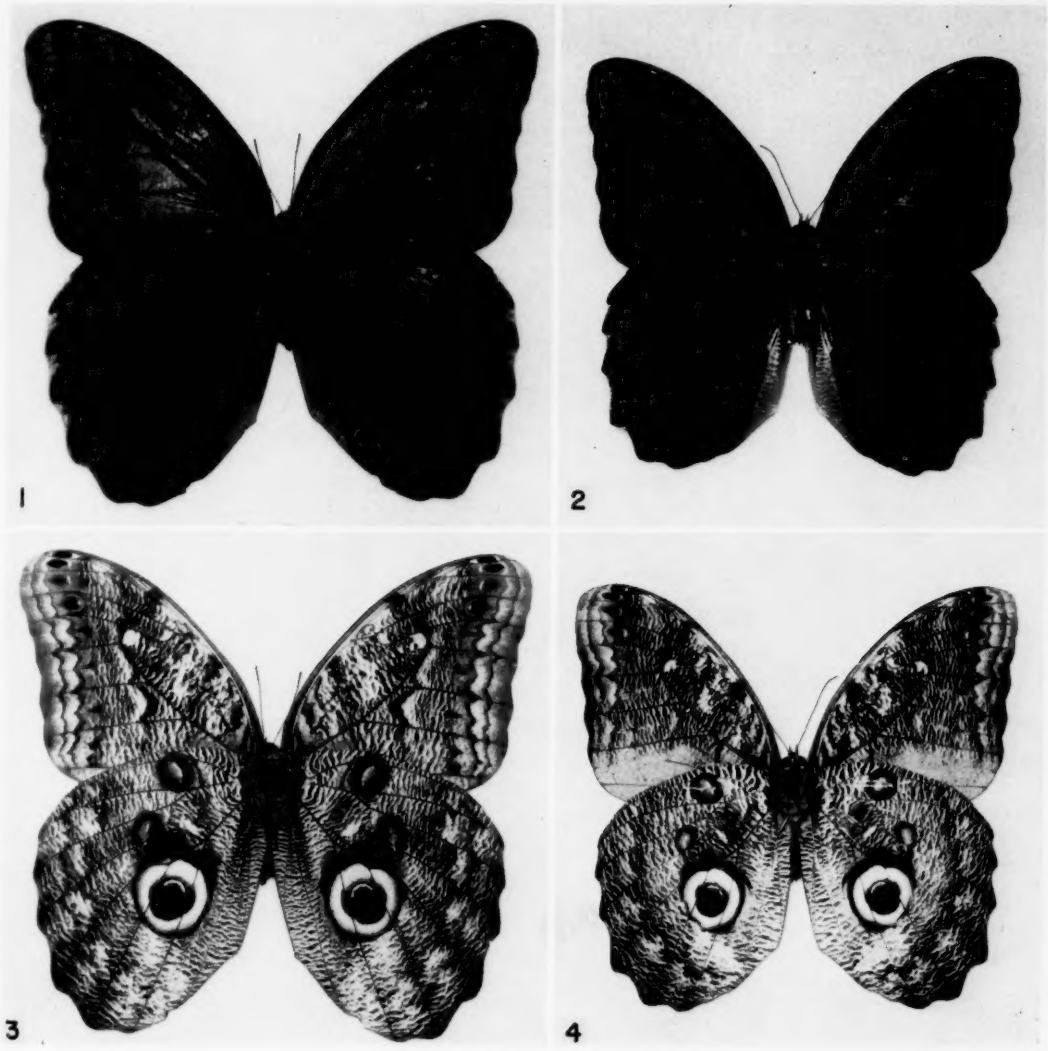
[2] A subsidiary of United Fruit Company.

[3] The authors thank the individuals cited in the text for determining specimens, and L. M. Roth, of these laboratories, for supplying data and for his interest and suggestions.

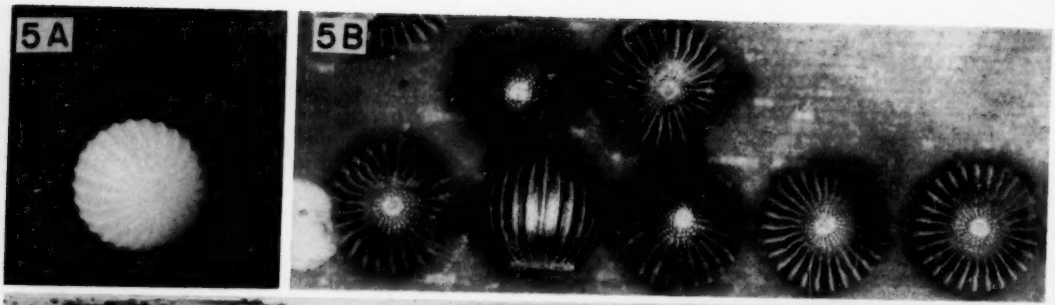


Adults of *Caligo eurilochus*, c.×0.5

FIG. 1.—Dorsal surface of female. FIG. 2.—Dorsal surface of male. FIG. 3.—Ventral surface of female. FIG. 4.—Ventral surface of male.

bears a lateral seta about midway along its length and a long terminal seta. Between the fourth and fifth days of larval life, a reddish-brown pigmented area, bearing a white spot posteriorly (on segment 4), develops on the dorsal abdominal surface of segments 3 and 4. Two smaller dorsal spots of the same color also appear at this time, one at the anal edge of segment 2, and the other at the anal end of segment 5 and the apex of segment 6. Four to 6 days after hatching, the larva displays on the dorsum of the thorax an area that first appears "water soaked," but which subsequently turns reddish brown except for two medial white streaks. This marking is retained in later instars (figs. 6, 9).

Second-Instar Larva.—Instead of dark setae, the head capsule now bears eight cuticular processes, which characterize the insect during the rest of its larval life and which become prominent horns in the later instars (figs. 6–9). However, in this instar only the two dorsal processes are dark and hornlike; the six lateral processes are light colored and become progressively much smaller ventrally. The predominant body color is a pale creamy or yellowish green with lateral stripes of light brown. Dorsal abdominal markings are similar to those described for the first instar. Four soft, dorsal abdominal spines appear in this instar on the anal edges of segments 2, 3, 4, and 5; the largest of these develops on segment 3. Usually the smallest spine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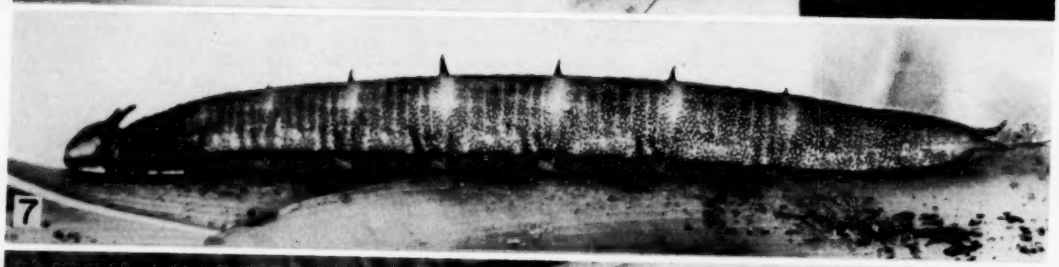
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can only be seen with the aid of a magnifying lens. In about a week the larva grows to about an inch in length and molts.

Third-Instar Larva.—The head now bears six definite horns, the remaining two ventrolateral processes being still only very small bumps. The two dorsal horns are large and black; the lateral horns are smaller and very light buff, almost white. Over-all body color is greenish yellow; the lateral brown stripes are wider than in previous instars, extending from the middle of the sides ventrally to the bases of the prolegs. In this species there are five pairs of prolegs, on segments 3, 4, 5, 6 and 10. In addition to the four spines of the second instar, a fifth dorsal abdominal spine is regularly present on the anal edge of segment 6; occasionally, a sixth spine is represented by a slight protuberance on the anal edge of the first abdominal segment. The four central spines are associated with the darkly pigmented areas described for the first-instar larva. By the end of this stadium the larva has grown to a length of $1\frac{1}{2}$ to $1\frac{3}{4}$ inches.

Fourth-Instar Larva.—The head now bears eight horns; all are nearly white except the two dorsal horns, which are striped with brown laterally from base to middle. All horns bear a few, fine, white setae. The frons has a pattern of vertical stripes, which are brown and tan (fig. 9). The body is brown; under low magnification the apparently solid color resolves into small maculations on a pale greenish background. Six dark-brown, dorsal spines are regularly present. By the end of this stadium the larvae are $2\frac{3}{4}$ to 3 inches long.

Fifth-Instar Larva.—This differs from the preceding instar chiefly in size and color. The head capsule (fig. 9) is very similar to that of the fourth instar. The general body color is dark brown, being made up of numerous dark maculations on a tan background. Eight prominent, light-tan, intersegmental bands mark the larva from the apex of the first abdominal segment to the eighth (figs. 6, 7). Between these relatively conspicuous bands are groups of six narrow, light-tan stripes. On the ventromeson of the prothorax, between the legs and head, there is a red, ventral eversible gland, similar to that figured by Peterson (1959, p. 115). By the end of this stadium the larva is $4\frac{3}{4}$ to 6 inches long and $\frac{1}{2}$ to $\frac{5}{8}$ of an inch in diameter at segment 4.

Pupa.—The pupa is about 2 inches long and $1\frac{3}{16}$ inches across its greatest width; it generally is light brown although color varies with the background against which the insect pupates. There are six patches of dark setae arranged longitudinally along the middorsal surface (figs. 17, 18). A few dark setae also occur dorsally on the head and last two abdominal segments.

LIFE HISTORY AND BEHAVIOR.—During the period May to September, 1957, when *C. eurilochus* was reared in the laboratory, the mean maximum daily temperature at Tenguel was $83.5 \pm 0.5°$ F. and the mean minimum daily temperature was $70.3 \pm 0.36°$ F. ($N = 118$).

Adult.—In the laboratory at Tenguel adults lived an average of 55 days (table 1). Adults survived less well at Norwood. L. M. Roth, of these laboratories, reared a few specimens from egg to adult. One male lived only 2 days after emergence; two females lived 13 and 43 days, respectively.

In the field the adult butterflies prefer shade when resting, but they feed in sun or shade on ripe or rotting banana fruit or on fresh manure. In banana plantations there appeared to be 8 to 10 times more males than females. Mating pairs generally rest on horizontal banana leaves, mainly on suckers, the two sexes facing in opposite directions; they remain in this position for long periods, scarcely moving.

Egg.—The eggs hatch in about 9 days (table 1). As many as 148 eggs have been removed from the ovaries of virgin females. Although virgin females will deposit unfertilized eggs, none of these have been observed to develop parthenogenetically.

Eggs are deposited mainly on the underside of the banana leaf, the upper part of the pseudostem, or at the base of the leaf petiole. Although a single egg may be deposited, usually the eggs occur in groups of from 3 to 20, groups of seven being the most common. The eggs are not deposited in a pattern; some occur in irregular groups, others in a single row which may be straight (fig. 5B) or curved. Prior to eclosion the larva perforates the shell and cuts a dorsal flap around the top of the egg with its mandibles; it pushes this flap aside when it leaves the shell.

Larvae.—Durations of larval instars are given in table 1. Larvae of *C. eurilochus* generally attack the second and third oldest leaves of banana plants that are about to shoot fruit or those already bearing fruit. Young leaves and small suckers adjacent to the main plant are rarely attacked unless the infestation is severe. Larvae attack the leaf from its tip or feed from the lateral edges toward the midrib. In heavy infestations leaves may be eaten completely to the midrib, and from three to five leaves per plant consumed, thereby considerably reducing the foliar surface. Feeding occurs at night. During the day young larvae rest in groups of up to 15 individuals on the undersurface of the leaf in the angle between the midrib

FIG. 5.—Eggs of *Caligo eurilochus* on banana leaf, c.×10. A.—Recently laid egg. B.—Shells of eggs that had been parasitized by *Xenufens* sp. (alcoholic specimens). An emergence hole made by the wasps may be seen on the right side of the bottom left egg. The second egg in the bottom row was turned on its side to show the flat base. FIGS. 6 and 7.—Fifth-instar, laboratory-reared larva of *C. eurilochus*, c.×1. In nature the pseudostem of the potted banana plant on which the larva rests was oriented vertically with the head of the larva up. FIG. 8.—Late-stage larvae of *C. eurilochus* in daytime resting place along midrib on under surface of field-collected banana leaf, c.×0.5. FIG. 9.—Larva of *C. eurilochus* that had been parasitized by *Apanteles opsiphanes*, c.×1. The mass of wasp cocoons has arched the abdomen of the larva away from the leaf. The larva was alive when photographed.

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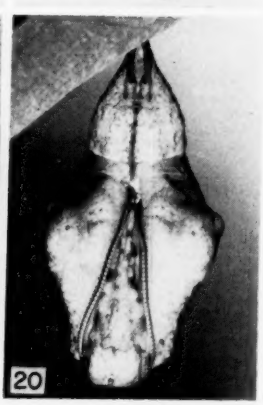
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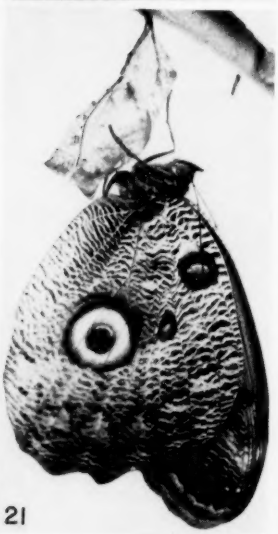
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and the blade. Older larvae may similarly rest on the leaves (fig. 8) or on the pseudostem close to the leaf petioles (figs. 6, 7). When changing position on the plant, the larva travels over a path of silk which it lays down before it while oscillating its head from side to side as it moves forward.

Pupa.—Duration of the pupal stage is given in table 1. The insect generally pupates on the ventral surface of the midrib of a banana leaf, but pupae occasionally hang from the edges of leaves or from stems of fruit.

About 3 days before pupation the larva enters a prepupal stage during which its length shortens to 3 or 4 inches. Having first spun a small network of silk on the pupation site, the larva remains stationary, parallel with the leaf for about 24 hours. It then relaxes the grip of its thoracic legs and that of the second, third, and fourth pairs of prolegs, retaining its grasp on the leaf with its first and fifth pairs of prolegs; thus the head and thorax bend down and the middle of the abdomen sags away from the leaf (fig. 10). After about 12 hours the prepupa releases its first pair of prolegs and hangs head down by the anal prolegs (fig. 11). In about 24 hours the insect pupates (figs. 12–16). The larval skin splits longitudinally along the dorsum of the thorax and, with head capsule attached, slips up over the anal end of the pupa (fig. 15); the exuvium drops free (fig. 16), leaving the pupa anchored by its cremaster to the silk button on the midrib. How the pupa is able to work the larval skin (the posterior end of which is an intact tube) between the cremaster and the leaf, without losing its grip and falling to the ground, is not known to us. The entire process, from the splitting of the larval skin to the shedding of the exuvium, may occur in as short a period as 4 or 5 minutes. At first the pupa is creamy white and elongated (fig. 16). Within 30 minutes it becomes shorter, approaching the final pupal shape (fig. 17); at this time the part of the chrysalis over the future wings is quite convex. By the end of 2 hours the pupa has assumed its final shape (figs. 18–20).

At eclosion the pupal skin usually splits dorsally along the grooves that are just mesal to the edges of the underlying wings (fig. 19), forming a "V." Ventrally the chrysalis splits along sutures over the antennae (fig. 20); these join and extend further posteriorly, forming an inverted "Y." The newly emerged adult hangs from the empty tan chrysalis (fig. 21) for several hours until its wings harden.

BIOLOGICAL CONTROL.—In Tenguel the immature stages of *C. eurilochus* are attacked by 8 parasitic insects and one predator. The action of these agents has undoubtedly caused the large fluctuations in the population of host insects that have been observed in this area. In fact, natural enemies have reduced the population of *C. eurilochus* to such a low level that it is not currently considered to be a serious pest. However, by its continued presence *C. eurilochus* remains a potential threat to the banana industry. If cultural practices should ever upset the natural balance between host and parasites, *C. eurilochus* might easily become an important pest.

Table 1.—Life-history data for *Caligo eurilochus*, reared on banana leaves in the laboratory at Tenguel, Ecuador.

STAGE	DURATION OF STAGE (DAYS)			NUMBER OF INSECTS
	Mean ± S.E.[a]	Minimum	Maximum	
Egg	8.9±0.26	7	10	15
1st instar	7.7±0.33	6	10	15
2d instar	7.5±0.40	6	10	15
3d instar	8.6±0.37	7	10	12
4th instar	9.7±0.46	7	12	13
5th instar	17.6±0.71	14	21	12
Pupa	18.1±0.29	17	20	15
Adult	54.8±3.2	40	78	12

[a] Standard error.

Egg Parasite.—The only parasite that has been reared from the eggs of *C. eurilochus* is the wasp *Xenufens* sp. near *ruskini* Girault (Trichogrammatidae) (determined by B. D. Burks, U. S. Department of Agriculture). This is one of the most important parasites of *C. eurilochus* in the Tenguel area. Over a 12-month period 82% of the eggs examined in nine farms were found to be parasitized (Malo, 1961). The normally white eggs turn gray then black after they have been parasitized, hence are easily recognized. The adult wasps emerge through a circular hole that they make in the shell near its apex (fig. 5B). As many as 20 to 40 *Xenufens* have been collected from a single *Caligo* egg. Further information on this association may be found in Malo (1961).

Larval Parasite.—The wasp *Apanteles opsiphanes* Schrottky (Braconidae) (determined by C. F. W. Muesebeck, U. S. Department of Agriculture) parasitizes the larva of *C. eurilochus*. Upon completing their development, the wasp larvae leave the host and spin a group of cocoons in a compact mass between the ventral surface of the host and the leaf (fig. 9). This mass of cocoons contains 7 to 12 rows of pupae, some rows having as many as 25 individuals; silk from the upper row of cocoons completely covers the host's prolegs, and the lowest row of cocoons is attached to the leaf. The parasitized *Caligo* larva remains alive until the adults of *Apanteles* have emerged, whereupon it either dies in place or breaks away from the mass of cocoons, but it never pupates.

Larval and Pupal Parasites.—Five species of flies parasitize the fourth- or fifth-instar larva of *C. eurilochus*, but

Pupation of *Caligo eurilochus* in the laboratory, c.×1.

FIG. 10—Prepupa clasping banana-leaf petiole, 0900 hours, 21 December 1959. In nature the petiole was oriented as in the following photographs. FIG. 11.—Prepupa has released the first pair of prolegs, 0800, 22 December. FIGS. 12 through 16.—Pupation, 0840 to 0850, 22 December. FIG. 17.—Pupa at 0915, 22 December. FIG. 18.—Pupa at 1115, 22 December. FIG. 19.—Dorsal view of pupa. FIG. 20.—Ventral view of pupa. FIG. 21.—Recently emerged adult, c.×0.5.

Table 2.—Results of surveys for larvae of *Caligo eurilochus*, killed by parasitic flies on banana farms, Tenguel, Ecuador.

Month	Farm: Esperanza Total Larvae	Esperanza Number and (%) Dead	Pichincha Total Larvae	Pichincha Number and (%) Dead	Balarezo Total Larvae	Balarezo Number and (%) Dead
			1957			
May	297	47 (16)	241	19 (8)	29	3 (10)
June	316	78 (25)	385	39 (10)	81	18 (22)
July	677	69 (10)	273	24 (9)	82	4 (5)
Aug.	492	95 (19)	105	17 (16)	32	0 (0)
Sep.	603	28 (5)	61	12 (20)	75	4 (5)
Oct.	199	21 (11)	33	9 (27)	44	2 (5)
Nov.	54	7 (13)	36	8 (22)	18	2 (11)
Dec.	33	6 (18)	39	6 (15)	19	1 (5)
			1958			
Jan.	15	3 (20)	2	1 (50)	4	0 (0)
Feb.	4	0 (0)	2	1 (50)	18	2 (11)
Mar.	14	1 (7)	6	0 (0)	20	0 (0)
Apr.	40	7 (18)	12	0 (0)	37	0 (0)
May	49	3 (6)	29	0 (0)	40	3 (8)
June	26	2 (8)	20	0 (0)	10	3 (30)
July	51	0 (0)	14	1 (7)	11	2 (18)

Table 3.—Results of surveys for pupae of *Caligo eurilochus*, killed by parasitic flies on banana farms, Tenguel, Ecuador.

Month	Farm: Esperanza Total Pupae	Esperanza Number and (%) Dead	Pichincha Total Pupae	Pichincha Number and (%) Dead	San Francisco Total Pupae	San Francisco Number and (%) Dead
			1957			
May	254	171 (67)	72	38 (53)	287	204 (71)
June	113	84 (74)	112	70 (62)	251	179 (71)
July	146	105 (72)	73	54 (74)	185	112 (60)
Aug.	524	372 (71)	120	69 (58)	335	225 (67)
Sep.	335	290 (87)	106	85 (80)	559	376 (67)
Oct.	223	187 (84)	39	31 (79)	148	104 (70)
Nov.	171	120 (70)	72	54 (75)	127	95 (75)
Dec.	77	56 (73)	55	42 (76)	64	46 (72)
			1958			
Jan.	48	48 (100)	14	13 (93)	83	59 (71)
Feb.	39	31 (79)	3	2 (67)	134	125 (93)
Mar.	23	9 (39)	18	14 (78)	103	86 (83)
Apr.	18	14 (78)	36	23 (64)	74	67 (90)
May	9	8 (89)	9	8 (89)	20	12 (60)
June	16	8 (50)	9	5 (56)	126	88 (70)
July	12	9 (75)	20	10 (50)	184	136 (74)
Aug.	10	2 (20)	20	12 (60)	47	32 (68)
Sep.			11	8 (73)	44	26 (59)
Oct.			13	7 (54)	41	19 (46)
Nov.			11	5 (45)	4	2 (50)
Dec.			20	8 (40)	17	11 (65)

the parasites generally do not complete their development until after the host has pupated. Adults of four species of flies, collected upon emergence from pupae of *C. eurilochus*, have been determined by C. W. Sabrosky, U. S. Department of Agriculture, to be *Sarcophaga* (*Sarcodexia*) *lambens* Wied., *Sarcophaga* sp. (possibly new) (Sarcophagidae), *Achaetoneura* sp., and *Winthemia* sp. (both Tachinidae).

Eggs of the above flies, species not determined, have been found mainly on the last seven abdominal segments of fifth-instar larvae; some larvae were found with as many as 28 eggs attached. These cream-colored eggs are 0.8 to 1.0 mm. long and 0.3 to 0.4 mm. wide. The newly hatched fly larvae enter the host and feed internally. Larvae of *C. eurilochus* that are parasitized by these flies usually complete metamorphosis and pupate, but occasionally the larvae die (table 2). When the fly larvae have consumed the contents of the pupa, they perforate the chrysalis and drop to the ground where they bury themselves under an inch of soil and pupate. From 6 to 26 larvae of the above four species of flies complete development within the host pupa. The typical, reddish-brown puparia measure approximately 6 by 2.5 mm. Adult flies emerge in 20 to 25 days; they have been seen around the farms feeding on ripe bananas.

Another fly, *Megaselia* sp. (Phoridae) (determined by W. W. Wirth, U. S. Department of Agriculture), attacks larvae of *C. eurilochus* in the fourth and fifth instars. In contrast to the relatively small numbers of sarcophagids and tachinids per host insect, a hundred or more *Megaselia* complete their development in the *Caligo* pupa; as many as 158 adults of *Megaselia* have been collected from one pupa. After consuming the contents of the pupa, the *Megaselia* larvae crawl onto its outer surface and pupate there. In the laboratory these fly puparia remained attached to the chrysalis until the adult flies emerged 10 to 15 days later. However, behavior may be different in the field, because *Caligo* pupae have never been collected with puparia of *Megaselia* attached.

The extent of control provided by all species of parasitic flies is shown in tables 2 and 3. At the time these surveys were made, the identity of the flies was not yet known, so no breakdown to species is possible.

Pupal Parasite.—Only one strictly pupal parasite of *C. eurilochus* has been found thus far; this is another fly, *Megaselia* sp. (determined by W. W. Wirth), which is recognizably different from the species of *Megaselia* that initially attacks the larva. From the small numbers seen, we infer that this fly is not particularly important in the Tenguel area.

Larval and Pupal Predator.—An as-yet-undetermined hemipteron (Reduviidae?), about $\frac{1}{2}$ to $\frac{3}{4}$ of an inch long, attacks larvae of *C. eurilochus* by inserting its beak into the first or second thoracic segment. Larvae so attacked throw their heads back as though trying to dislodge the predator with their head horns. Occasionally, the bug shifts its attack to the first or third abdominal segment and continues feeding. A day or so after such an attack, the host tissue turns black around the wound. However, the bug may continue feeding until the host dies; the dead larva collapses like an empty bag but remains attached to the leaf, from which it hangs like an inverted "U," suspended by its abdominal prolegs, or it may hang head down, attached by its anal prolegs.

This bug also attacks the pupa and sucks out the contents, leaving the chrysalis empty.

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Time, Temperature and Dosage Relationships of Several Insecticidal Fumigants[1]

EUGENE E. KENAGA, *The Dow Chemical Company, Midland, Michigan*

ABSTRACT

A laboratory study was made of the relationships of temperature, exposure time and dosage with the insecticidal action of several space and commodity fumigants. The adult confused flour beetle, *Tribolium confusum* Jacquelin duVal, was the test insect. The fumigants tested were carbon disulfide, sulfuryl fluoride, acrylonitrile, methyl bromide, ethylene dibromide, carbon tetrachloride, methyl chloroform, ethylene dichloride and chloropicrin. The LD-50, LD-95 and confidence limits were determined for each of the above fumigants at 2-, 5- and 16-hour exposure periods and at temperatures of 40°, 60° and 80° F. (4.44°, 15.56° and 26.67° C.). Development of prediction formulas are discussed. With few exceptions, it was found that dosage requirements increase with a decrease in temperature or exposure time. The LD-95 values give essentially straight lines when plotted as graphs on log-log paper using the products of dosage-time, dosage-temperature or time-temperature as the y coordinate and time, temperature or dosage as the x-coordinate. The slope of the equations of the various products of the fumigants is of a similar magnitude regardless of chemical structure or of insect species.

The ability to predict insecticidal dosage requirements of commercial fumigants under varying conditions of time and temperature has long been desired. Mortality data for nine fumigants at three temperatures and three exposure periods are given in this paper. These data are used to develop formulas, the application of which allows one to interpolate dosages for intermediate temperature and exposure conditions. Temperatures and exposures were chosen to encompass a wide range of practical fumigation conditions. The information presented here concerns space fumigation and is not discussed in relation to commodity fumigation where additional sorption due to the commodity increases the dosage required to maintain lethal concentrations.

FUMIGANT MATERIALS.—The fumigants chosen for study represent a variety of chemical structures and several known modes of insecticidal action. Also, materials such as ethylene dibromide (EDB-1,2-dibromoethane), ethylene dichloride (EDC-1,2-dichloroethane), acrylonitrile (VCN-vinyl cyanide), chloropicrin (CP-trichloronitromethane) and methyl bromide (MB-bromomethane) at some 100% lethal dosages have no visible anesthetic or lethal effect immediately after the fumigation, but may cause increasing daily mortality 2 to 4 weeks following fumigant exposures. Fumigants such as carbon tetrachloride (CCl_4), 1,1,1-trichloroethane (MC-methyl chloroform) and sulfuryl fluoride (SO_2F_2) may have little or no lingering post fumigation effects on treated insects. Carbon disulfide (CS_2), unlike the other fumigants, has an anesthetic effect on insects at low sublethal dosages from which they may recover completely.

The fumigants used in this study were of a purity of 99% or greater.

TEST INSECTS AND ENVIRONMENTS.—A number of insect species have been used in insecticidal fumigation studies; however, stored product insects are most commonly favored as being indicative of commercial problems. The confused flour beetle, *Tribolium confusum* Jacquelin duVal (CFB), is an insect with medium to high resistance to fumigants. It is a long-lived insect with desirable laboratory rearing and handling characteristics. It is also an important worldwide pest of stored grain and other commodities. The life stages of the confused flour beetle are found to be of different resistance to various fumigants, one fumigant being more toxic to the adult, another to the egg stage, et cetera. However, the adult stage appears to be the most representative of life stages and was used throughout this investigation. It was reared on a mixture of 90% whole wheat flour and 10% dried Torula yeast, at a temperature of 80° F. The test insects were adults of mixed sexes, 4 to 8 weeks old. They were preconditioned to the desired test temperatures of 40°, 60° or 80° F. for 3 hours to 3 days before fumigation and were held at 80° F. (plus or minus 1° F.) for 2 weeks after fumigation prior to making mortality counts. The relative humidity during fumigation varied from 40% to 90%. During the pre- and postfumigation period it varied from 60% to 90%. Under these conditions essentially no natural mortality occurred.

The low temperature of 40° F. was a limiting factor for preconditioning or postconditioning periods because the test organism did not tolerate exposures to this temperature for more than 5 days without appreciable mortality. Fourteen days of conditioning at this temperature resulted in complete mortality either in or out of the insect media. At 60° F. using methyl bromide at sub-lethal dosages, it was determined that there were no significant mortality differences between preconditioning the insects for 2 hours or for 3 days.

Fumigation temperatures of 40°, 60° and 80° F. were chosen as representatives of conditions under which commercial fumigations might occur. It was recognized that 40° F. is cold enough to cause almost complete cessation of movement of the confused flour beetle adult. However, such a temperature does commonly occur in commercial storages and, for the purposes of this study, fumigant-insect interactions at this temperature were of interest. Exposure periods of 2, 5 and 16 hours represent several time periods of commercial fumigant exposures.

The effects of various fumigants and fumigation conditions on insect mortality as reported by Reynolds (1948–58), Sun (1947), Pradhan & Govidan (1953–54) were considered and used in standardizing insect handling and holding methods. It was concluded by an analysis of all sources available that the test methods used and

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Table 1.—Time, temperature and dosage relationships for nine fumigants.

t HOURS	°T ° F.	d = (MG./L.) LD-50	LD-95	±95% C.L
		A. Methyl bromide		
16	80	3.60	4.64	0.63
5		9.57	11.84	1.61
2		22.68	27.90	4.09
16	60	5.05	5.80	0.79
5		17.24	23.21	4.22
2		41.28	55.02	10.51
16	40	6.64	13.08	2.04
5		26.71	37.93	8.75
2		90.75	145.14	45.56
		B. Ethylene dibromide		
16	80	0.745	0.924	0.206
5		1.47	1.90	0.11
2		2.61	3.36	0.53
16	60	0.64	0.88	0.12
5		1.80	2.34	0.22
2		3.97	5.70	0.98
16	40	0.97	1.47	0.30
5		2.33	5.60	1.89
2		6.20	8.90	1.62
		C. Carbon tetrachloride		
16	80	57.71	76.58	11.57
5		106.03	165.22	48.81
2		209.34	302.10	52.96
16	60	78.81	108.42	22.86
5		201.26	344.50	71.07
2		351.41	521.81[a]	58.80
16	40	252.90	412.51[a]	88.20
5		483.88[a]	896.90[a]	255.42
2		634.64[a]	1,085.46[b]	247.53
		D. Methyl chloroform		
16	80	52.78	72.07	14.28
5		121.70	188.84	33.53
2		262.93	409.33	70.54
16	60	84.69	121.84	33.83
5		221.82	353.72	84.20
2		356.26	519.62[a]	101.41
16	40	208.32	326.31	74.17
5		399.88[a]	703.09[a]	19.25
2		620.92[a]	1,139.96[b]	190.39
		E. Ethylene dichloride		
16	80	4.03	5.80	1.13
5		9.95	15.21	3.53
2		18.07	36.78	19.20
16	60	7.72	21.13	10.20
5		19.92	39.51	12.11
2		48.98	87.90	18.35
16	40	9.19	26.16	12.90
5		24.50	76.38	39.32
2		62.53	125.45	32.84
		F. Chloropicrin		
16	80	1.94	2.79	0.44
5		4.64	6.29	0.58
2		11.23	14.86	1.78
16	60	3.37	4.84	0.95
5		9.28	11.12	0.98
2		23.14	33.49	5.27
16	40	2.18	7.03	3.52
5		8.59	20.90	7.29
2		21.63	39.69	7.22
		G. Acrylonitrile		
16	80	1.36	1.66	0.27
5		2.83	3.30	0.22
2		6.88	8.46	1.27
16	60	1.52	1.91	0.29
5		3.83	5.22	0.82
2		8.95	11.63	1.46
16	40	2.27	3.80	1.47
5		8.35	13.75	2.63
2		11.93	27.37	11.71
		H. Carbon disulfide		
16	80	29.95	33.68	5.02
5		69.79	100.33	18.09
2		176.30	227.47	36.75
16	60	42.09	60.21	11.18
5		126.93	173.98	29.60
2		224.22	271.03	38.91
16	40	119.75	165.62	27.83
5		250.71	380.13	67.24
2		511.06	737.34[b]	120.07
		I. Sulfuryl fluoride		
16	80	3.88	4.72	0.45
5		8.70	11.09	1.23
2		14.10	16.89	2.10
16	60	5.35	6.10	0.68
5		11.36	14.42	2.95
2		22.54	29.60	6.17
16	40	7.53	11.14	2.00
5		22.45	42.12	9.26
2		86.61	117.54	16.33

[a] Super-saturation dosage.
[b] Dosage not actually obtained.

reported in this paper represent conditions allowing maximum survival of the insects and therefore constitute a rigorous test.

TEST EQUIPMENT AND FUMIGANT APPLICATION.—The fumigation chambers used in these tests were 25.5-liter aluminum pressure cookers whose inner surfaces were treated with a baked-on coat of Heresite. The covers to these vessels were precision ground and, when treated with a thin coating of silica grease, held a 30-inch vacuum. The covers were equipped with multiple entry ports, two of which were fitted with $\frac{1}{8}$-inch diameter pipes and controlled with valves through which fumigants were introduced and vacuum attained. Another inch diameter port was fitted with a rubber stopper through which the test insects were introduced and withdrawn. A coarse 4-mesh stainless steel screen rack was installed in the chamber and served to hold the caged insects near the center of the

chamber space. Insect test cages were made from ¾-inch outside-diameter polyethylene tubing, cut into 2-inch lengths. These cages were stoppered at each end with corks and a series of ventilating slits were cut lengthways by use of a fine jewelers'-disk saw. These cages allowed ample ventilation and space for the insects. During exposure each cage contained 33 adult insects. Three cages were placed in the fumigation chambers. When using liquid fumigants the cages were placed in the chambers prior to application of the vacuum.

The liquid fumigants were weighed in a ventilated hood by use of small Erlenmeyer flasks with an attached side arm stop-cock valve and with a top stoppered with neoprene rubber which was fitted with a stop-cock valve as illustrated by Kenaga (1953). Weights were to the nearest 0.1 mg. on an analytical balance. The fumigants were introduced by evacuating the chamber to a 25-inch vacuum and then fastening the Erlenmeyer flask containing the fumigant to the chamber via rubber tubing, opening the flask and vault valves and sweeping the fumigant along with outside air into the chamber until atmospheric pressure was achieved. When necessary, the flask was gently warmed to aid in complete volatilization of the fumigant. A thermometer was inserted in the cover of the fumigation chamber so as to observe the occasional initial change in temperature. This change was in the order of 1 to 2 degrees Fahrenheit due to warming of the flask.

Gaseous fumigants (methyl bromide and sulfuryl fluoride) were measured with a 100-ml. gas burette, which was equipped with a plunger and a three-way glass valve and Luer-Lok fittings. These were easily connected to and disconnected from the chamber. A positive filling of the burette was assured by gaseous pressure directly from the fumigant pressure cylinder. On application of the fumigant, each stroke of the plunger caused a positive displacement of the gas from the burette. Release to atmospheric pressure was accomplished by opening the three-way stop-cock valve at the end of the gas burette. With completion of the gas application the chamber was returned to atmospheric pressure and the fumigant was allowed to diffuse for 2 to 3 hours to reach equilibrium at the testing temperature before introducing the insects.

Upon termination of the exposure period the chambers were opened and the insects were immediately placed into holding containers and provided with food and aeration. Insect mortality counts were made after 14 days in a holding room. Mortality was determined by initially removing all obviously live insects and subjecting the remainder to a controlled electric hot plate to which live insects reacted. Insects which reacted in any way to the hot plate were considered alive.

Fumigation dosages are expressed in terms of milligrams per liter which is numerically equivalent to ounces per 1,000 cubic feet.

RESULTS AND DISCUSSION.—The dosage-mortality responses for each fumigant and each temperature-time unit, *i.e.*, 80° F.—16 hour exposure, 80° F.—5 hours exposure, and so forth, were plotted on log-probability paper. Dosage responses between 1% and 99% mortality are quite linear on log-probability paper from which the LD-50 and LD-95 may be estimated by eye. However, this method was not used for analysis. The data were also fitted to the equation: mortality (y) equals intercept (a) plus slope (b) times dosage (x) by the methods of least squares. Of course most of the values for 0% and 100% mortality had to be discarded. The LD-95 values from these two methods differed by no more than 4.6% and were generally within 1%. There was little or no difference in the LD-50 but rather large differences in the LD-1 and LD-99.

Since there was this excellent agreement and since no factors of judgement as to where to draw lines were involved and because the lay reader can visualize this straight line formula more readily, the data of table 1, A–I inclusive, were calculated by this latter method.

Lindgren & Vincent (1959) and Kenaga (1956) graphically illustrate the sorption of various fumigants on fumigation chamber surfaces and on various commodities and at varying temperatures. Most of the sorption takes place in the first few minutes of exposure and tends to reduce the concentration of the fumigant in the air space. Apparently very little is known about the amount of sorption which takes place on or in the insect. This sorption may be entirely different from sorption on the container or in and on the commodity being fumigated. Sorption thus far discussed has been at unsaturated vapor dosages. In table 1, C, D, and H it is demonstrated that air saturation dosages of carbon tetrachloride, methyl chloroform and carbon disulfide were sub-lethal to insects under several short exposure and/or low temperature conditions. When more fumigant was introduced than needed to saturate the atmosphere it was observed that the mortality responses continued as a straight line function just as though no saturation point had been reached. Under these conditions the fumigation chamber and the insects were sometimes visibly moist with the fumigant at the end of the exposure period. Because the air concentration remains the same at saturation while insect mortality increases with further dosage increase, the term "dosage" is used instead of concentration throughout this paper, though in most cases dosage (d) and concentration are essentially equivalent as determined by representative checks of air samples. A formula published by Roark & Nelson (1929) was used to calculate fumigant concentrations which may exist in air. Using this formula, the approximate saturation dosages, converted to mg. per liter, are as follows:

Fumigant	4.44° C. 40° F.	15.56° C. 60° F.	26.67° C. 80° F.
CCl_4	373	479	598
MC	371	476	5 2
CS_2	682	862	1,036

Greater than saturation dosages are starred in table 1, C, D, and H.

Kenaga (1957) published on the effect of various temperatures and exposure periods on the insect toxicity of methyl bromide and sulfuryl fluoride. The data were obtained from fumigation techniques similar to those discussed here. An exception is that the 1957 fumigation results were obtained from the use of different gas application equipment, and the insects were placed into the chamber *before* the vacuum was taken prior to applying

Table 2.—Simple correlation coefficients[a] of dosage (d), time (t) and temperature (°T) and products.

Coordinates										
y	x	MB	EDB	CCl_4	MC	EDC	CP	VCN	CS_2	SO_2F_2	Average
t	°T	0.10	0.08	0.12	0.10	0.09	0.51	0.09	0.22	0.18	0.17
°T	d	.55	.46	.61	.39	.29	.59	.37	.59	.48	.48
t	d	.55	.44	.35	.60	.53	.66	.64	.73	.52	.56
t°T	d	.56	.10	.53	.66	.53	.66	.64	.77	.53	.55
d°T	t	.67	.43	.56	.63	.60	.69	.70	.85	.63	.64
dt	°T	.78	.44	.64	.55	.39	.43	.48	.69	.61	.56

[a] At the 99% confidence level a simple correlation coefficient of 0.30 or greater is significant.

the fumigant. This change in procedure may account for the slightly higher dosages needed in the 1960 tests to obtain equivalent mortality responses. When the 1957 and 1960 LD-95s are plotted on the same log-probability paper for 40°, 60° and 80° F., the lines are parallel, indicating a close relationship.

DEVELOPMENT OF PREDICTION METHODS.—E. M. Reynolds and co-workers (1948–58) have reported on the use of concentration-time (ct) products using methyl bromide, ethylene dibromide, ethylene dichloride, carbon tetrachloride, hydrogen cyanide, ethylidene chloride, trichloroethylene and tetrachloroethylene at several temperatures and using various insect species. Brown (1959) has made use of the ct relationship to illustrate concentration requirements for methyl bromide under four different temperatures. Magnitudes of differences in the ct products, on different species, of 2 to 3 times were frequent; 5 times not uncommon, but 10 times was exceptional. Differences between life stages were as great as between fumigants.

The author has experienced similar magnitudes of differences in similar situations. In spite of these differences, it appeared as though there might be something in common which would be useful in the interpolation of fumigant dosages between known dosage responses. To be most useful, the interpolation would have to include the variables of time of exposure (t), temperature (°T) and dosage (d) of the fumigant. It would be desirable to find a method to graph these responses so that the result would be a straight line and therefore easily interpolated.

Table 3.—Correlation coefficients[a] of dt and d°T products plotted on rectangular coordinate and log-log paper. (d equals LD-95)

	Coefficients When Plotted on			
	Rectangular Coordinate Paper		Log-log Paper	
Fumigant	dt (°T)	d°T (t)	dt (°T)	d°T (t)
MB	0.90	0.82	0.96	0.96
EDB	.85	.78	.88	.87
CCl_4	.77	.88	.88	.95
MC	.80	.80	.89	.93
EDC	.76	.73	.65	.88
CP	.93	.70	.88	.88
VCN	.90	.79	.95	.93
CS_2	.86	.82	.94	.96
SO_2F_2	.80	.87	.86	.94
Average	.84	.80	.88	.92

[a] At the 99% confidence level a simple correlation coefficient of about 0.40 or greater is significant.

An effort was made to find a relationship among the variables by multiple correlation using a complete quadratic model. The many elements of each matrix used in these correlation studies are too many to present in this paper; however, the following conclusions are made:

1. No single equation for all fumigants which would have equal terms could be found.
2. In no cases were the second order terms significant.
3. No evidence of sampling bias due to time, temperature, test method, and so forth, was found.
4. It was evident that there was a positive relationship among the variables time, temperature and dosage.

The simple correlation coefficients of dosage, time and temperature, and products are given in table 2. These results are presented solely to help select the most promising correlations for further study. All three correlations were significant; however, the d°T (t) (product of dosage times temperature as related to time) correlation proved to be the highest (0.64 average) with the dt (°T) and t°T (d) correlations being lower (0.56 and 0.55 averages, respectively). The d°T (t) and dt (°T) relationships were selected for further work; the first because it gives the highest correlation and the second because the similar ct product has been previously used in the literature.

The next step necessary for graphing of the variables was the selection of a specific dosage-mortality response for each fumigant. The LD-95 was chosen to represent d because it approximates the amount needed for a commercial dosage without stretching the accuracy of the data as would an LC-99.9. There were 81 LD-95 values used, or nine for each fumigant based on the three time and temperature variations for each of the nine fumigants.

The type of paper to be used for graphing the dt (°T) and d°T (t) relationships was the object of the next evaluation. Using the LD-95s for each fumigant, the various time and temperature products respectively were plotted on both rectangular and log-log paper. Reasonably straight lines were obtained on the log-log paper, especially if temperature was expressed in °C. The exception to this rule is chloropicrin which plotted best on rectangular paper. A mathematical confirmation of these graphical findings was needed. The linear formula, $y=a+bx$, was again used where y=the cross products, a=intercept, b=slope and x=the third variable. Temperature was expressed in °C., time in hours and the LD-95 in mg. per liter. Both arithmetrical and log figures were calculated by the machine and the correlation coeffi-

Table 4.—Log values used in prediction formulae for dosage-time products *dt* (°T).

FUMIGANT	INTERCEPT	SLOPE	STANDARD ERROR OF *dt*
MB	2.817	−0.697	0.075
EDB	1.706	−0.512	0.092
CCl_4	4.163	−0.856	0.171
MC	4.004	−0.705	0.132
EDC	3.023	−0.814	0.348
CP	2.362	−0.531	0.107
VCN	2.221	−0.665	0.073
CS_2	3.842	−0.790	0.107
SO_2F_2	2.646	−0.651	0.141

To calculate *dt* (°T) coordinate relationships, fill in the following formula:
$d \times t$ = intercept plus (slope × ° C) in log figures

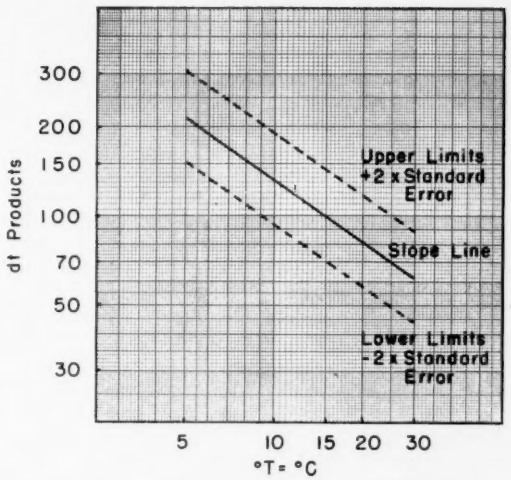
cients shown in table 3. These data confirm the superior results obtained by the use of log-log paper over rectangular in most cases and the slight advantage of the *d*°T (*t*) correlation over the *dt* (°T) on log-log paper. By the use of these data, formulas are calculated which can be used to graph the slope and standard errors of each fumigant. The formulas for the *dt* (°T) relationships of the nine fumigants are shown in table 4 and the *d*°T (*t*) relationships are shown in table 5. Graphs drawn from formulas presented in these tables can be used by the fumigator to fit variables of time or temperature, whichever is needed for his immediate work. Graph No. 1, illustrated by the use of methyl bromide, shows such relationships based on the *dt* (°T) formula. For the purpose of showing variability, the slope line plus or minus two times the standard error of the cross products are shown. The area between these two lines includes about 95% of the total cross product variables. The 16-hour *dt* product line appears to be approximately one to two standard errors above the slope or 5-hour *dt* line while the 2-hour *dt* line appears about one to two standard errors below this slope. Likewise, the graphed data from table 5 would show the 80° F. *d*°T product line to be approximately one to two standard errors above the slope or 60° F. *d*°T line and the 40° F. *d*°T line to be about one to two standard errors below.

Only nine LD-95 points are available from table 1, A–I inclusive, for each fumigant; however, intermediate *dt* products for carbon disulfide, acrylonitrile, ethylene dichloride and ethylene dibromide for intermediate tem-

Table 5.—Log values used in prediction formulae for dosage temperature products *d*°T (*t*).

FUMIGANT	INTERCEPT	SLOPE	STANDARD ERROR OF *d*°T
MB	3.170	−1.031	0.134
EDB	2.116	−0.824	0.180
CCl_4	4.052	−0.634	0.090
MC	4.116	−0.716	0.122
EDC	3.189	−0.776	0.177
CP	2.764	−0.853	0.194
VCN	2.523	−0.899	0.127
CS_2	3.890	−0.776	0.087
SO_2F_2	2.937	−0.837	0.125

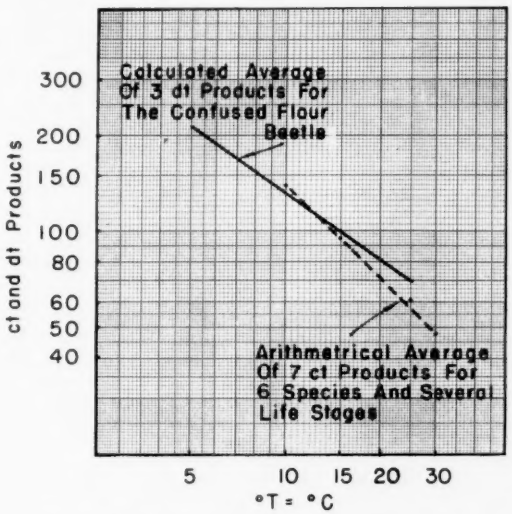
To calculate the *d*°T (*t*) coordinate relationships, fill in the following formula:
$d \times$ ° C = intercept plus (slope × time) in log figures



GRAPH NO. 1.—Methyl bromide *dt* (°T) relationships based on formula from table 4.

peratures of 50° F. and 70° F. all fall within the same correlation pattern of plus or minus two times the standard error shown in tables 4 and 5.

So far the variables of time, temperature and dosage have been discussed on the basis of the reaction to one life stage of one insect species. It is noted that each fumigant has its own characteristic average behavior which can be tabulated or can be illustrated graphically on coordinates to predict dosage requirements under varying conditions within the scope of this study. What happens to the predictability when applied to other species of insects and their life stages? Brown (1959) reports data using methyl bromide on a variety of 11 stored product



GRAPH NO. 2.—Comparison of slope lines of methyl bromide using data from Brown and Kenaga.

beetle species and their various life stages. Estimates of the minimum concentration-time (*ct*) products using the LC-99.9 are given for 10°, 15°, 25° and 30° C., apparently based on a range of time-concentration responses. When the *ct* product responses of the various species are plotted against temperature on log-log paper, they form lines, some straight, some curved and some with irregular shapes, similar to the irregular responses obtained by the author on previously described individual *dt* products. Brown's LC-99.9 *ct* products line for the confused flour beetle adult graphs nearly parallel and lies very close to the author's LD-95 *dt* products line for the same species. The averages of Brown's 1959 *ct* products for all species with data at 10°, 15°, 25° and 30° C. were plotted on log-log paper. These average points formed a straight line which is nearly parallel to the confused flour beetle *dt* lines. (See graph 2.) Thus, it is seen that generally most insect species and life stages tested respond similarly to temperature and exposure fluctuations within the limits of the variables expressed in this paper. Surprisingly, all fumigants studied here have slopes which are in the same order of magnitude regardless of chemical structure or mode of action.

Considerable experimental variation is found in determining the resistance of different species and life stages of insects to the various fumigants under the many time-exposure conditions which may be used in commercial fumigations. It is not easy to predict conditions which can be used with a guarantee of 100% mortality and minimum time and dosage for known temperatures.

The graphical method of showing the inter-relationship among these variables is an oversimplification from a statistical standpoint and is not rigorous in this respect. However, these data showing the general relationships among the variables on a single graph should help in the selection of the dosage for the fumigator's particular fumigation conditions. Suitable compensating factors should be employed which take into consideration the fact that some life stages of insects may be more resistant than those used in this work and that the predictions are only average expectations. The use of the graph for fumigation dosage prediction is aided by the selection of a few known commercially successful dosage-time-temperature points which can be placed with confidence on a graph (calculated from the formulas in table 4) such as graph No. 1. The selection of dosages for a new set of fumigation conditions can be made by drawing a line parallel to the calculated lines but passing through the known successful *dt* (°T) points for a similar fumigation experience.

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Effects of Insecticides on Insects and Pathogenic Fungi Associated with Alsike Clover Roots[1]

C. M. Leach, E. A. Dickason, and A. E. Gross[2]

Abstract

Field studies were conducted in Oregon in an attempt to determine if a relationship existed between clover root curculio (*Sitona hispidula* (Fabr.)) injury to the roots of alsike clover and the incidence of pathogenic fungi. Soil insecticide treatments of aldrin, heptachlor, and DDT were applied during the spring of 1957. During the autumns of 1958 and 1959 seed yields were obtained and roots were sampled and rated for the incidence and severity of both insect injury and disease. There were no differences in seed yields between treated and untreated plots in 1958, but in 1959, all treatments resulted in significantly increased yields over the untreated plots. All the treated plots showed less larval injury of the roots during 1958, and all except those treated with DDT during 1959. There was also a corresponding significant reduction of vascular root decay in aldrin-treated plots during 1958, and in aldrin- and heptachlor-treated plots during 1959. During both years some diseased plants showed no insect injury and, conversely, some healthy plants had been injured by insects.

The fungi most frequently isolated from diseased roots were *Fusarium oxysporum* Schl. sensu Snyd. and Han., *Verticillium albo-atrum* Reinke & Berth., and an undescribed species of *Phoma*. All three species are pathogenic to alsike clover, but only species of *Fusarium* are known to be assisted in host penetration by insect wounds.

Alsike clover, *Trifolium hybridum* L., has long been the principal leguminous crop grown for seed in the Klamath Basin of Oregon. Within recent years the longevity of stands and yields of seed have declined. Plants have invariably shown severe root deterioration which has been attributed to two causal agents, insects and fungi.

Injury by the clover root curculio, *Sitona hispidula* (Fabr.), is the most conspicuous symptom associated with deterioration. The root system is damaged by larvae feeding on root nodules, on small secondary fibrous roots, and by their gnawing deep cavities in the tap root. This type of injury to legumes was first reported by Wildermuth (1910) and Webster (1915).

The principal symptoms of disease are vascular discoloration of the root stele, cortical decay, and rotting of rootlets. The fungi most frequently isolated from diseased roots are *Fusarium oxysporum* Schl. sensu Snyd. and Han. (Snyder & Hansen 1943), an undescribed species of *Phoma* (Hardison 1952), and *Verticillium albo-atrum* Reinke and Berth., all of which have been proved pathogenic to alsike clover by Corden (1955).

Several workers have suggested on the basis of field observations that there is a possible relationship between wounding of clover roots by the clover root curculio and the incidence of root rots caused by pathogenic fungi (Jewett 1934, Elliott 1952, Underhill *et al.* 1955, Kilpatrick & Dunn 1958). Such a relationship, however, has never been established experimentally under field conditions. Graham & Newton (1959, 1960) in greenhouse experiments established a positive relationship between root feeding by the clover root curculio and clover root borer, *Hylastinus obscurus* (Marsh.), and the incidence of root rots of red clover and Ladino white clover caused by *Fusarium roseum* (Lk.) Snyd. and Hans., and other *Fusarium* spp. (species not cited).

During earlier clover root curculio control studies (Dickason *et al.* 1958), the writers observed that vascular decay of alsike clover frequently originated at the site of insect injury (fig. 1) and it was thought that the insect wounds might be increasing the incidence of disease. An experiment was therefore designed to determine if controlling the clover root curculio would result in a corresponding reduction of diseased plants, and an increase in yield of seed and longevity of stand.

Procedure.—Experiments were conducted at the Klamath Branch Experiment Station in Klamath County, Oregon, an irrigated area of low rainfall having hot summers and freezing winter temperatures.

Insecticide plots were established during April, 1957, in an attempt to control larvae of the clover root curculio as they moved through the soil to the root region of the plants. Each plot was 31 by 182 feet and each treatment was replicated four times. All insecticides were emulsifiable concentrate formulations applied in 10 gallons of water per acre and disced into the soil immediately following application. The treatments and rates of actual toxicants per acre were: DDT, 5 lbs.; aldrin, 2 and 4 lbs.; and heptachlor, 2 and 4 lbs. The area was seeded to oats following treatment and alsike clover was drilled into the oat stubble in late summer.

Plants were sampled during the autumns of 1958 and 1959. Fifty plants were obtained from each plot by digging 5 plants at each of 10 randomized locations. Plants were washed and the roots examined for insect injury and disease, and rated as follows: 0 = no insect injury or disease, 1 = slight, 2 = moderate, 3 = severe, and 4 = plant dead. Plants from each treatment were grouped into insect injury categories, and these groups were then rated on the incidence of vascular discoloration and cortical decay. Isolations were made from roots showing typical vascular discoloration by plating surface sterilized portions of roots on streptomycin (200 p.p.m.) potato-dextrose agar.

Seed yields were obtained by harvesting a 3-foot swath through each plot during the autumns of 1958 and 1959.

The experiment was originally planned to continue through at least three harvest seasons. However, the study was abandoned after the second seed-crop year

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[2] Assistant Plant Pathologist and Assistant Entomologist of Oregon State College, Corvallis, and Associate Agronomist, Oregon Agricultural Experiment Station, Klamath Falls, respectively.

FIG. 1.—Longitudinal section of alsike clover root showing vascular discoloration caused by *Fusarium oxysporum* originating at site of insect injury (see arrows).

because of increased insect injury in treated plots due to breakdown of insecticides, and because germination of shattered and unharvested seed resulted in a heterogeneously aged stand.

RESULTS AND DISCUSSION.—*Insect Injury.*—The effect of insecticides on clover root curculio injury is summarized in table 1. All treatments significantly reduced

Table 1.—The effect of insecticides on insect injury of alsike clover roots during 1958 and 1959.

TREATMENT AND ACTUAL TOXICANT PER ACRE (LB.)	INSECT INJURY INDICES (MEANS)[a]		PER CENT OF ROOTS INJURED (MEANS)	
	1958	1959	1958	1959
Untreated	1.29	2.64	78	96
Heptachlor, 2	0.38	1.22	18	60
Heptachlor, 4	0.27	1.15	5	60
Aldrin, 2	0.54	2.03	36	86
Aldrin, 4	0.49	1.96	25	85
DDT, 5	0.78	2.48	51	96
L.S.D. @ 5% level	0.38	0.40		
@ 1% level	0.53	0.55		

[a] Injury index: 0 = no injury, 1 = slight, 2 = moderate, 3 = severe and 4 = plant dead.

Table 2.—The effect of insecticides on vascular root decay of alsike clover during 1958 and 1959.

TREATMENT	DISEASE INDICES (MEANS)[a]		PER CENT ROOTS DISEASED (MEANS)	
	1958	1959	1958	1959
Untreated	0.92	1.28	43	65
Heptachlor, 4 lb.	0.71	0.75	34	37
Aldrin, 4 lb.	0.48	0.83	27	43

	Analyses[b]	
	1958	1959
Untreated vs. treated plots	significant (5%)	significant (1%)
Untreated vs. heptachlor, 4 lb.	not significant	significant (1%)
Untreated vs. aldrin, 4 lb.	significant (5%)	significant (1%)
Heptachlor vs. aldrin	not significant	not significant

[a] Disease index: 0 = no disease, 1 = slight, 2 = moderate, 3 = severe, and 4 = plant dead.

[b] Analyses based on number of plants infected rather than per cent or disease indices.

the amount of larval injury to the roots during 1958, and all but DDT during 1959. In the first year (1958), the percentage of roots injured in heptachlor- or aldrin-treated plots was very low in contrast to untreated plots. However, in the second year (1959), these chemicals had apparently lost much of their effectiveness in controlling larvae, resulting in an increase in percentage of roots injured.

Under field conditions, the decline of alsike clover is most apparent during the second seed crop year. It is evident from the insect injury indices (table 1) that there is a marked increase in severity of insect injury during the second crop year. Since there was reseeding from shattered and hard seed throughout the experiment, all of the 1959 injury was not necessarily the additive effect of 2 years' insect activity.

Root Disease.—The incidence and severity of root disease in the treated and untreated plots are summarized in table 2. Disease readings were based only on vascular discoloration because cortical decays were negligible. The rating was restricted to untreated plots, aldrin (4 lbs.), and heptachlor (4 lbs.) treated plots. During 1958 there was significantly less vascular root decay in aldrin-treated plots than in heptachlor or untreated plots. During 1959, roots from both aldrin- and heptachlor-treated plots showed significantly less vascular decay than plants from untreated plots. On the basis of plot treatments, these results suggest that where insect injury was significantly reduced (table 1) there was also a cor-

Table 3.—Fungi isolated from surface disinfected roots of alsike clover plants.

FUNGI ISOLATED	PER CENT OF PLANTS INFECTED[a]	
	1958[b]	1959[c]
Fusarium oxysporum	65	63
Fusarium spp.	8	9
Phoma sp.	41	73
Rhizoctonia solani	—	2
Saprophytes	—	5
Unidentified spp.	—	2
Verticillium albo-atrum	38	2

[a] Many plants were infected with more than one pathogen.

[b] Isolations from 105 plants.

[c] Isolations from 545 plants.

Table 4.—Relationship of insect injury (grouped by classes) to the incidence of root disease.

Insect Injury Class	1958 Samples[a]						1959 Samples[b]					
	Disease Class				Disease Indices	Total Plants	Disease Class				Disease Indices	Total Plants
	0	1	2	3			0	1	2	3		
	(number of plants)						(number of plants)					
0	58	27	21	14	0.93	120	77	11	14	15	0.71	117
1	63	22	18	17	0.91	120	37	16	10	6	0.60	89
2	45	30	18	27	1.23	120	77	15	11	16	0.71	119
3	40	33	20	27	1.28	120	100	53	58	63	1.31	275

[a] Correlation coefficient significant at 1% level.
[b] Correlation coefficient not significant.

respondingly significant reduction in the incidence of diseased roots (table 2). This does not necessarily indicate that the incidence of disease is directly related to insect injury.

Table 3 lists the fungi isolated from surface disinfected roots. The three species most commonly isolated were *Fusarium oxysporum*, an undescribed *Phoma* sp., and *Verticillium albo-atrum*, all of which were proved pathogens of alsike clover (Corden 1955). A relationship between insect wounding and the incidence of fusarial root rots has been established under greenhouse conditions (Graham & Newton 1959, 1960). No similar relationship has been established for *Verticillium* or the undescribed species of *Phoma*.

Relationship of Insect Injury to Root Disease.—An attempt was made to correlate insect injury and the occurrence of diseases in individual plants. In the winter of 1958, plants were sampled from an untreated plot until 120 plants were grouped in each of the insect injury categories (0 through 3) and the individual plants were then scored for root discoloration. The analyses of these data showed a positive correlation between insect injury and the presence of vascular decay (table 4). In spite of this statistically positive correlation, there were plants which showed no insect injury that were diseased, and conversely, there were plants with insect injury that were not diseased. Undoubtedly some injury, particularly feeder-root pruning, could not be detected by visual examination at sampling time, and it is possible that the zero (uninjured) category may have included some injured plants.

Insect injury was so severe in the untreated plots during 1959 (table 1) that it was impossible to sample sufficient plants for inclusion in each of the previously mentioned categories. Consequently, the 1959 data presented in table 4 were based on plants from both treated and untreated plots. Analyses of these results showed no correlation between insect injury and the incidence of vascular decay.

An attempt was made during 1959 to isolate fungi from roots grouped under the different insect injury categories to determine if infection by specific pathogens was aided by insect injury. Unfortunately, because of inadequate surface disinfection of roots, not all surface fungi were killed, and since the origin of these fungi could not be determined, the data are questionable and are not included in the discussion.

Seed Yields.—Seed yields for 1958 and 1959 are presented in table 5. There were no significant differences during 1958 between treated and untreated plots, but during 1959 there was a significant difference between untreated and all treated plots.

CONCLUSIONS.—Paralleling the decline of alsike clover stands there was an increase of root deterioration caused by the larvae of the clover root curculio and by root decay fungi. Applications of insecticides to the soil reduced populations of curculio larvae, reduced incidence of root decay, and increased seed yield. Since insecticides were the only chemicals employed in the experimental design, it was impossible to satisfactorily determine whether increased seed yields were primarily a result of: 1) a reduction of insect injury, 2) a reduction of root rots because of reduced insect injury, 3) the combined reduction of insect injury and root rots. These results suggest that the decline is caused by a biotic factor, or complex of biotic factors, which are affected by insecticides, rather then by direct cultural, climatic, or nutritional factors.

The positive correlation between insect injury and the incidence of disease which was found after the first seed harvest would support the greenhouse findings of Graham & Newton (1959, 1960). In addition, the reduced numbers of diseased plants in the insecticide-treated plots in both years suggest a relationship between insect injury and infection by pathogens. It is our opinion that the positive correlation obtained between insect injury and the incidence of disease presents an overly simplified picture, for there were plants which were free of insect injury that were diseased and plants that were injured and free of disease. Of the three pathogens isolated from vascular tissue, only species of *Fusarium* are known to be assisted in penetrating host plants through insect wounds. Thus,

Table 5.—Yields of alsike clover seed in 1958 and 1959.

Treatment and Toxicant per Acre (lb.)	Yield in Pounds per Acre (means)	
	1958[a]	1959
Untreated	138.26	72.9[b]
Heptachlor, 2	132.14	104.0
Heptachlor, 4	129.61	119.5
Aldrin, 4	131.63	107.6
Aldrin, 4	126.52	121.9
DDT, 5	115.12	116.6

[a] No significant differences.
[b] Significant at 5% level between treated and untreated plots.

it is possible that reduced insect injury may have reduced the incidence of *Fusarium* root decay, but not root decays caused by *Verticillium* or *Phoma*. If this relationship existed during this study, it would explain the lack of correlation obtained during the second year.

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DDT Spray Formulations and Dosages for Control of Corn Stem Weevil, *Hyperodes humilis*, and Fall Armyworm, *Laphygma frugiperda*, on Sweet Corn[1]

EMMETT D. HARRIS, JR., *Florida Everglades Experiment Station, Belle Glade*[2]

ABSTRACT

Five sprays applied at 4-day intervals beginning at seedling emergence reduced the degree of infestation by the corn stem weevil, *Hyperodes humilis* (Gyll.), and the fall armyworm, *Laphygma frugiperda* (J. E. Smith) and increased the yield of sweet corn ears. Incidence of insect attack decreased as the dosage was increased from 1 to 3 pounds per 100 gallons and emulsions were more effective than wettable powder sprays. After weekly sprays that were applied later, 1.0 and 1.5 pounds of DDT per 100 gallons of emulsion were about equal but much more effective than 0.5 pound for fall armyworm control, and emulsions were more effective than wettable powder sprays.

When the corn stem weevil, *Hyperodes humilis* (Gyll.), was first recognized as a sweet corn pest on organic soils in the Everglades, it was observed that toxaphene or DDT sprays recommended for control of budworms (fall armyworm, *Laphygma frugiperda* (J. E. Smith) and corn earworm, *Heliothis zea* (Boddie)) did not effectively control this pest. Therefore, insecticides were initially tried at about double the dosage at which they were effective against most other pests (Harris 1960). Emulsions of 2 pounds of actual DDT per 100 gallons were effective when applied seven times at 4-day intervals, beginning the day of seedling emergence. Among the questions that remained unanswered were: is a DDT wettable spray as effective as an emulsion, is a "double" dose of toxaphene equal to one of DDT, and what is the minimum dosage of DDT that will provide effective corn stem weevil control?

Harrison & Harris (1958) reported that 1 pound of DDT per 100 gallons was more effective in an emulsion than in a wettable powder spray for control of the fall armyworm in the whorls of sweet corn plants. Harris (1959) compared DDT and toxaphene emulsions that contained 0.5, 1.0, 1.5, or 2.0 pounds of actual toxicant per 100 gallons in weekly applications and reported that a dosage greater than 1.5 pounds of either insecticide did not seem profitable.

The purpose of this study was to compare DDT emulsions with DDT wettable powder sprays, three dosages of each formulation, and a single toxaphene dosage with the DDT dosages for control of the corn stem weevil and budworms and the effect on stand and yield.

METHODS AND MATERIALS.—As a lower dosage is required for control of budworms than for corn stem weevil, it seemed desirable to use lower dosages after sprays were no longer necessary for control of the corn stem weevil. Therefore, upon completion of the corn stem weevil program, the dosage was halved at each level and treatments reassigned to plots so that each treatment for budworms followed each corn stem weevil treatment once. Dosages are shown in pounds of actual toxicant per 100 gallons in the appropriate figures. For observations taken before initiation of budworm sprays, the experiment was of randomized complete block design. After

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initiation of budworm sprays it became a Latin square design with corn stem weevil treatments and budworm treatments homologous to rows or columns in relation to each other. Thus, the effect of budworm treatments on comparison of corn stem weevil treatments, and vice versa, was minimized. Treatments were compared by single degrees of freedom analysis (Federer 1955).

Sixty-Pak sweet corn was planted October 2, 1959 in rows that were 36 inches apart and ran from north to south. Upon seedling emergence 20-foot alleyways were disked across the rows to form four tiers of plots containing rows that were 40 feet long. Each tier contained two blocks of eight plots. Plots were four rows wide and separated by single unsprayed buffer rows which the sprayer straddled in making applications.

Sprays were applied through Spraying Systems Disc-Type Teejet D4-23 hollow cone nozzles at 250 p.s.i. using a custom-made self-propelled small-plot sprayer (Harrison *et al.* 1959) equipped with a two-row outrigger boom. The rate of travel was approximately 2.4 m.p.h. The first spray was applied on October 9, 1 day after the seedling emergence. The next was applied on October 13 and was followed by three additional sprays at 4-day intervals. The first 4 sprays were applied with 2 overhead nozzles at 50 gallons per acre. On the fifth and final application on October 21, an additional nozzle on each side of the row directed spray under the foliage canopy to the lower stem and adjacent soil and the rate of application was 100 gallons per acre.

DDT and toxaphene emulsifiable concentrates contained 2 and 6 pounds of actual toxicant per gallon, respectively, and a xylene range aromatic hydrocarbon solvent. The wettable powder contained 50% actual DDT.

Sprays against budworms were applied through two overhead nozzles and an additional nozzle on each side of the row on November 4 and 11 at 100 gallons per acre. Beginning on November 18, 1 day after the first silks appeared, in all plots spray was directed toward the ear zone at 2 pounds of DDT in 50 gallons of emulsion per acre for control of the corn earworm. Sprays were applied at 4-day intervals until the silks were dry.

RESULTS.—*Corn Stem Weevil Control.*—Ten plants were taken from each of the two middle rows in every plot on

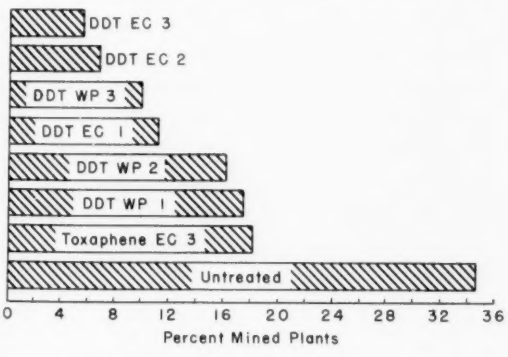

FIG. 1.—Effect of corn stem weevil sprays on per cent of mined plants.

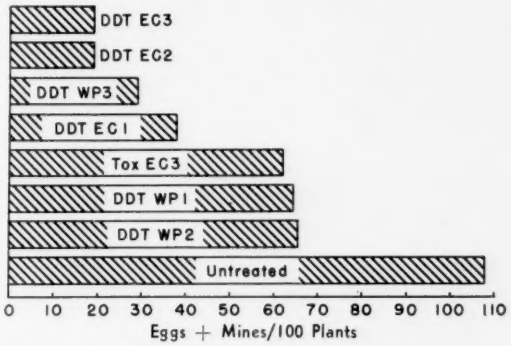

FIG. 2.—Effect of corn stem weevil sprays on number of eggs and mines per 100 plants.

October 22 and stored at 40° F. They were examined from October 23 through November 3 for the numbers that were egg infested and mined and the total numbers of eggs and mines per sample. Per cent egg-infested or mined plants, eggs or mines per 100 plants, or eggs plus mines per 100 plants differed little as criteria for comparing treatments. Per cent mined plants and eggs plus mines per 100 plants are shown in figures 1 and 2, respectively.

None of the comparisons among insecticide treatments were significant, but treated plots were highly significantly less infested than untreated ones.

Although insecticide treatments were not significantly different, there was a tendency for populations of the corn stem weevil to decrease as dosage increased with the exception of 1 and 2 pounds of DDT in wettable powder sprays, which were nearly equal. Also, there was a definite tendency for the DDT emulsion to be more effective than DDT wettable powder or the toxaphene emulsion.

Spray Burn.—On October 22, spray burn occurred in some of the plots. Each plot was scored for the severity of this injury on October 23. None of the damage appeared to be more severe than slight and the following scoring system was used: 0) none, 1) very slight, 2) slight. All of the plots treated with wettable powder sprays or DDT emulsion at 1 pound per 100 gallons as well as the untreated check had no injury. DDT emulsion containing 3 and 2 pounds of actual toxicant per 100 gallons had average scores of 1.8 and 0.9, respectively. Toxaphene emulsion received a score of 0.3.

Budworm Control.—Budworm populations were light until about the time of the final corn stem weevil spray. Of 54 budworms collected from unsprayed buffer rows on November 2, there were 53 fall armyworms and 1 corn earworm. The extent of budworm damage was evaluated on November 2, 10, and 17 by counting the plants with fresh damage in the whorl among 25 plants in each of the two middle rows of each plot.

Corn Stem Weevil Treatments.—On November 2, among both wettable powder sprays and emulsions, the highest DDT dosage resulted in significantly fewer budworm-infested plants than the lowest dosage. There was not a significant quadratic regression of number of budworm-infested plants on dosages of DDT (fig. 3).

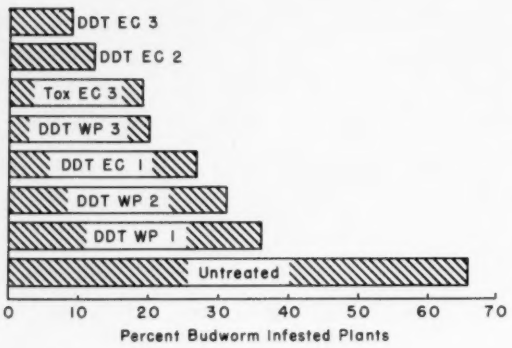

FIG. 3.—Effect of corn stem weevil sprays on per cent of budworm-infested plants.

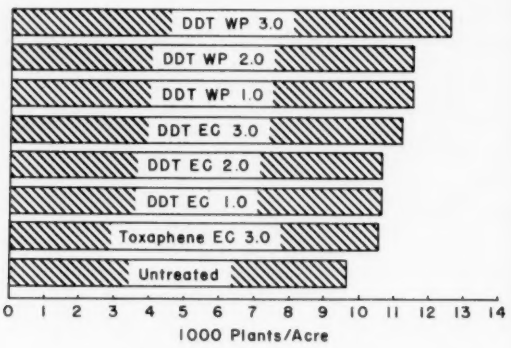

FIG. 5.—Effect of corn stem weevil sprays on stand of sweet corn.

DDT emulsions resulted in highly significantly fewer budworm-infested plants than the wettable powder sprays. Treated plots had highly significantly fewer budworm-infested plants than the untreated ones.

On November 10, 3 pounds of DDT per 100 gallons was still significantly more effective than 1 pound. DDT emulsion was significantly more effective than wettable powder spray, and insecticide treatments, as a group, significantly reduced the budworm infestation.

On November 17, there were no significant differences among the corn stem weevil treatments.

Budworm Treatments.—On November 10, there were no significant differences among insecticide treatments but as a group they resulted in a significantly lower budworm population than that in the untreated plots (fig. 4).

On November 17, there was a significant quadratic regression of budworm-infested plants on DDT emulsion dosages (fig. 4). DDT emulsion was highly significantly more effective than wettable powder sprays. Insecticide treatments resulted in highly significantly fewer budworm-infested plants than the untreated checks.

Stand Counts.—The plants in each of the two middle rows of each plot were counted on October 12 and November 20. On October 12, there were no significant differences among treatments. Treatment means ranged from 15.2 to 16.6 thousand plants per acre.

On November 20, there were no significant differences among the plots treated for control of the corn stem weevil, but as a group they resulted in a significantly greater stand than that recorded in the untreated check (fig. 5). There were no significant differences in stand among the plots receiving budworm treatments.

Yields.—The weight of U. S. Fancy and total marketable ears (U. S. Fancy and Nos. 1 and 2)[3] were taken to the nearest 0.1 pound in the two middle rows of each plot on December 16, 1959. These weights were converted to crates per acre using an approximation of 40 pounds per crate. Yields, even among the better treatments, were poor, probably as a result of cold and wet weather during the growth of this corn. Effect of treatments against the corn stem weevil are shown in figure 6. As a group, insecticide treatments highly significantly increased the yields of both total marketable and U. S. Fancy ears. There were no significant differences among the insecticide treatments. Among the budworm treatments there were no significant comparisons, and yields from treated plots were not significantly different from those recorded from untreated plots.

[3] U. S. Standards for Green Corn, May 18, 1954.

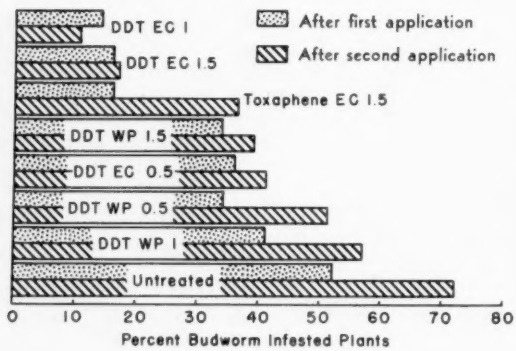

FIG. 4.—Effect of budworm sprays on per cent of budworm-infested plants.

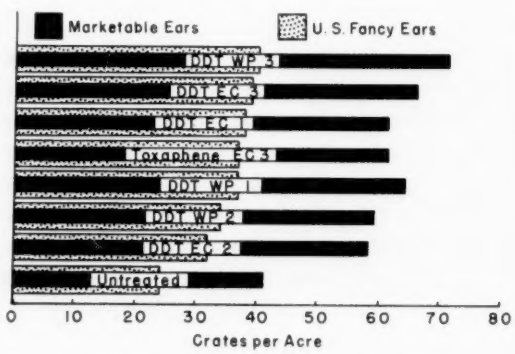

FIG. 6.—Effect of corn stem weevil sprays on yields of sweet corn.

DISCUSSION.—Failure to show significant differences among treatments for control of the corn stem weevil may have been the result of limitations in experimental precision rather than a real absence of treatment differences, especially with respect to the degree of corn stem weevil infestation and any effect of this insect on stands and yields. As DDT seemed to be effective mostly through destroying the adult before oviposition but not immediately upon contact (Harris 1960), migration among plots would tend to equalize the effects from the insecticide treatments. Such migration would be more critical in the 4-row plots used in this experiment than in the center of larger plots or in commercial fields. The single unsprayed rows that were left between plots may have had a bearing on this problem. Plot size should be less critical in evaluating budworm control as the insecticide treatments appear to act more on the caterpillar in the whorl in which stage it is less likely to migrate among plots.

Use of a relatively small sample of 20 sweet corn plants per plot was necessary because of the time required to make detailed observations on total numbers of corn stem weevil eggs and mines. Perhaps, a larger sample and less detailed observations would provide greater experimental precision. In this experiment, observations on the numbers of egg-infested or mined plants seemed to be as effective in detecting treatment differences as more detailed observations on the total numbers of eggs, mines, or eggs plus mines among the same plants.

Control of the corn stem weevil had a greater effect than budworm control on stand and yield, but the data did not indicate whether this resulted from reduction in populations of corn stem weevil or budworms. Observations during the growth of the crop indicated that loss in stand probably resulted from attack by the corn stem weevil rather than from budworms.

The results did not indicate need for a change in the recommendation of emulsions containing 2 pounds of actual DDT per 100 gallons for control of corn stem weevil. DDT wettable powder at 3 pounds of actual toxicant per 100 gallons did not appear to be as effective as the recommended dosage of emulsion and should be evaluated at even greater dosages. Although 3 pounds of actual DDT in a wettable powder spray provided yields that were higher than the other treatments, yields might be considerably decreased by a heavier population of the corn stem weevil.

At the high dosages used for control of the corn stem weevil, there was a residual effect on budworm populations as long as 20 days after the final application. This fact suggests the desirability of studying dosages in relation to intervals between applications for budworm control. Those treatments applied for budworm control did not appear to be very effective when applied against the established budworm infestation. Treatments against budworms may have resulted in much better control had they been applied throughout the growth period of the crop. The data indicated that dosages of 1 and 1.5 pounds of actual DDT per 100 gallons of emulsion were nearly equal in effectiveness, and indicated little need for increased dosages of emulsions except to increase the interval between applications. DDT wettable powder sprays, and possibly toxaphene emulsions, should be tried at dosages greater than 1.5 pounds of actual toxicant per 100 gallons for budworm control.

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INDEX XVII

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Effects of Temperature and Photoperiod on Voltinism of Geographical Populations of the European Corn Borer, *Pyrausta nubilalis*[1]

STANLEY D. BECK and JAMES W. APPLE, *University of Wisconsin, Madison*

ABSTRACT

A study was made of the effects of temperature and photoperiod on the developmental patterns of European corn borer population samples from northern Wisconsin, southern Wisconsin, southern Minnesota, Iowa, southern Ontario, Massachusetts, Nebraska, northern Kansas, Pennsylvania, and southeastern Missouri. Under a series of standard laboratory conditions of temperature and photoperiod, the incidence of diapause and rate of larval growth differed significantly among the several populations. The incidence of diapause was positively correlated with the latitude of the population source. The characteristics of the temperature and photoperiodic responses differed among the populations, indicating complex evolutionary trends. The populations differed not only in the effects of temperature and photoperiod on the incidence of diapause, but also in the effect of temperature on larval growth rates. The importance of these population differences in the voltinism manifested under field conditions is discussed. An empirical basis for predicting summer pupation of the borer in southern Wisconsin was developed, employing seasonal temperature accumulation up to a day length of 14.75 hours (July 25, at 43° N.).

Shortly after the discovery of the European corn borer, *Pyrausta nubilalis* (Hbn.), in the United States, it was observed that its seasonal biology in Massachusetts was different from that found in the Lake Erie region of New York. The Massachusetts population tended to produce two generations annually; whereas the New York borers tended to produce but one. As early as 1920, experiments were conducted to determine the extent of differences in voltinism (Caffrey & Worthley 1927). It was found that Massachusetts borers reared in a field insectary in New York persisted in a bivoltinism over the experimental period of 4 years. New York borers reared under similar conditions in Massachusetts continued to display univoltinism. The two populations could interbreed and produce viable progeny, however, and it was concluded that they represented geographical races of a single biological species.

The life history charts compiled by Caffrey & Worthley (1927) showed that a small per cent of the bivoltine Massachusetts population failed to pupate in midsummer, but went into diapause. Similarly, Barber (1925) observed a variable degree of bivoltinism in the typically univoltine New York borer population. The results of these two workers indicated the possibility that the expression of voltinism might be modified by climatic conditions, at least to a limited degree.

The European corn borer was found in New Hampshire in 1921 (O'Kane & Lowry 1927). Both univoltine and bivoltine characteristics were manifested by this population. O'Kane & Lowry observed that eggs hatching prior to about July 20 produced bivoltine larvae; later larvae were uniformly univoltine.

By 1921, the borer had also invaded Ohio and Michigan, where it appeared to produce but one generation per year (Pettit 1925, Caffrey & Worthley 1927.) The insect invaded Indiana about 1926, where it was also typically univoltine (Ficht 1936). Caffrey & Worthley (1927) pointed out, however, that a small second generation was occasionally observed in Ohio, Michigan, and Indiana. In 1931, the borer was found in Wisconsin, typically univoltine (Chambers 1941). The borer was first discovered in Illinois in 1939, and has typically shown a mixed voltinism in which about half of the first generation larvae enter diapause. The corn borer reached Iowa in about 1941 (Harris & Brindley 1942), where it has been typically bivoltine. The borer was first reported in Missouri in 1942 (Vance 1942), and it reached Minnesota (univoltine) in 1943 (Holdaway *et al.* 1949). Its presence was reported in Kansas, Nebraska, and Tennessee in 1944 (Vance 1945), and 1946 brought its invasion of North and South Dakota (Beck, E. W. 1947). By 1950, the borer had been reported as far south as Mississippi, and westward into Oklahoma and Colorado (Beck, E. W. 1951).

Because of the economic importance of the insect, interest has been shown in identifying the biological and ecological factors influencing the year-to-year expression of voltinism. Babcock (1927, a, b) expressed the Lamarckian opinion that a particular seasonal cycle that had been impressed on a borer population for several years would tend to persist, even if the borers were transferred to different environmental conditions. Babcock also considered univoltinism to be the optimal condition for the species. O'Kane & Lowry (1927), working in New Hampshire, observed that a large proportion of second generation larvae usually failed to mature sufficiently to survive the winter. These workers concluded that bivoltinism was inherent in the population and was not a response to the length of the growing season. They considered the bivoltine race poorly adapted to New Hampshire conditions.

The supposedly univoltine borer populations of Michigan, Ohio and Indiana began to display a distinct trend toward bivoltinism in about 1934 (Ficht 1936, Vance 1939). Subsequently, an increasing tendency to produce two generations per growing season has been observed in other parts of the corn-growing regions of the midwest, including Ontario (Vance 1947, Bradley 1947, Wressel 1953, Miller 1957). Vance (1939) and Arbuthnot (1944) found that bivoltine borers grew more rapidly under controlled temperature conditions than did univoltine borers. Arbuthnot (1944) demonstrated that univoltinism acted as a recessive genetic character. He isolated an apparently homozygous univoltine strain, but was unable to isolate a

[1] Approved for publication by the director of the Wisconsin Agricultural Experiment Station. This study was supported in part by a research grant from the National Institutes of Health of the U. S. Public Health Service. Accepted for publication December 9, 1960.

pure bivoltine line. He also found that moths of the two strains did not interbreed readily, although he obtained some fertile matings.

On the basis of annual mean temperature and precipitation records, Arbuthnot (1949) attempted to account for the voltinism observed in different geographical areas. He divided the United States into a series of zones based on climatic prediction of voltinism. Such zonation has neither proved useful nor provided a sound basis for year-to-year prediction of the extent of midsummer pupation in the northern half of the corn belt. Using a developmental threshold of 50° F., Apple (1952) devised a temperature accumulation basis for the prediction of the seasonal phenology of the different growth stages of the borer. The method has not proved useful in predicting the extent of summer pupation, except that it has been observed that above normal temperature accumulations early in the growing season tended to be followed by a large second generation (Chiang & Hodson 1959).

Mutchmor & Beckel (1958, 1959) and Beck & Hanec (1960) demonstrated that the induction of diapause in the borer is dependent on photoperiod as well as temperature, thus adding day length as an ecological variable in the voltinism problem. These studies clearly showed that diapause is facultative, and that the insect is a "long day" species; that is, pupation occurs under long photoperiods and diapause under short photoperiods. Some doubt, thereby, has been cast on the applicability of the terms "univoltine" and "multivoltine." It would seem likely that differences in the threshold of response to diapause-inducing conditions could account for observed differences in voltinism. The experimental borer populations employed by Beck & Hanec (1960) showed a range of photoperiodic sensitivity that approximated a normal distribution. The finding was interpreted as being consistent with the genetic results of Arbuthnot (1944), and as an indication that the borer population studied was heterozygous for multiple genetic factors controlling the photoperiodic response.

Mutchmor (1959) demonstrated that midsummer pupation of the borer under field conditions was closely associated with day length. Under Ontario conditions, he found that borers reaching maturity after day lengths (sunrise to sunset) shortened to less than 14.5 hours entered a state of diapause. Borers attaining the fifth instar prior to this time pupated. Beck & Hanec (1960) showed that the diapause-inducing effect of photoperiod was temperature-sensitive. High rearing temperatures tended to reduce the diapause incidence under short photoperiods.

Temperature and day length conditions differ among different areas of the corn borer's North American distribution, depending on locale and latitude. The adaptation of the borer to particular local conditions might, by a process of natural selection, involve changes in the frequencies of genetic factors controlling photoperiodic responses. The present study was undertaken to test this hypothesis by measuring diapause incidence under controlled laboratory conditions, using borers from several geographical locations.

MATERIALS AND METHODS.—The European corn borer larvae used were progeny of overwintering borers collected in the autumn and winter of 1959–60. Borers collected from the area of Madison, Wisconsin were taken from a very late field of sweet corn infested only with second generation borers. Mature larvae or pupae derived from other overwintering populations were provided by a number of cooperators, to whom we are greatly indebted. Population samples from Minnesota, Kansas, Iowa, and Pennsylvania were supplied by Dr. T. A. Brindley of the U. S. Department of Agriculture and Iowa State University. Dr. J. H. Lilly, University of Massachusetts, collected a sample of Massachusetts borers. Dr. C. C. Burkhardt, Kansas State University, furnished a second group of borers from Kansas. Missouri specimens were supplied by Dr. M. L. Fairchild, University of Missouri, and a Nebraska sample was obtained through Dr. G. T. Weekman, University of Nebraska. Mr. P. W. Smith, Wisconsin Department of Agriculture, helped supply borers from Wausau, Wisconsin. Dr. H. B. Wressel, Canada Department of Agriculture, provided a sample of Ontario borers.

Temperature correlations were determined on the basis of normal daily mean temperatures, furnished by the National Weather Records Center, Asheville, North Carolina. Day lengths were calculated from astronomical tables, and were taken as the number of hours from sunrise to sunset, with no allowance for morning and evening twilights.

Laboratory Methods.—Newly hatched unfed borer larvae from the several populations were reared aseptically on purified diets according to the technique of Beck & Smissman (1960). Exposure to photoperiods and the measurement of diapause incidence was by the experimental procedure of Beck & Hanec (1960).

Comparisons of the temperature and photoperiodic responses of the different borer populations were made on the basis of larval growth rates and diapause incidence under a series of standard conditions. The standard conditions employed were:

(1) 14.5 hour photoperiod (14.5 hr. light, 9.5 hr. dark per 24 hr.) at 26° C.
(2) 14.5 hour photoperiod at 30° C.
(3) 15.0 hour photoperiod (15.0 hr. light, 9.0 hr. dark per 24 hr.) at 26° C.
(4) 0 hour photoperiod (continuous dark) at 26° C.

This experimental design allowed comparison of response to two photoperiods (14.5 and 15.0) at a constant temperature (26° C.) and to two temperatures (26° and 30° C.) at a constant photoperiod (14.5). Continuous dark (treatment 4) served as a control to demonstrate photoperiodic responses where normal photoperiods might fail to elicit response differences. Borers under non-photoperiodic conditions normally display little or no diapause.

From 400 to 550 newly hatched larvae were set up from each population. The larvae were distributed randomly among the four treatments described above. As the larvae reached maturity, daily pupation records were taken. Diapause incidence was measured as the per cent of mature larvae that failed to pupate. This was determined on the basis of the sigmoid configuration of the pupation record (see figure 1); when the curve

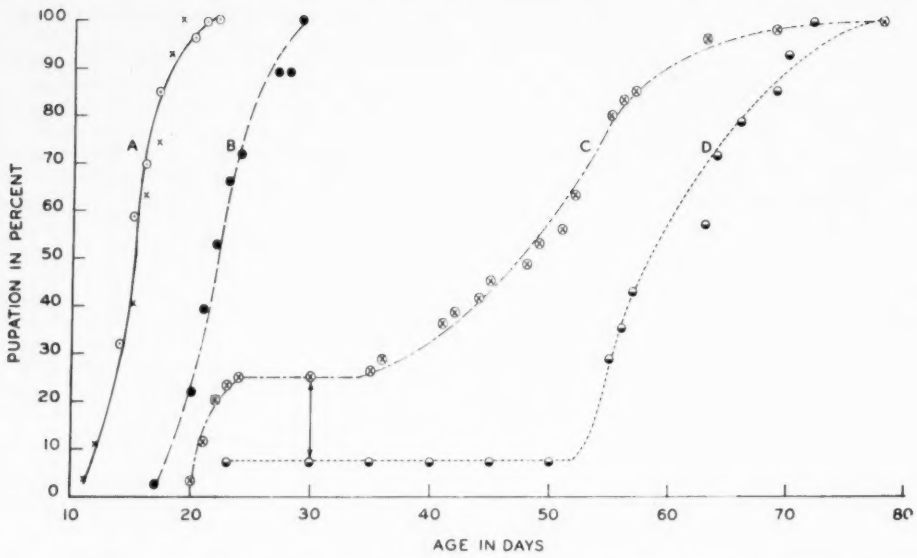

FIG. 1.—Pupation curves of Ontario and Wisconsin European corn borer larvae under different conditions of temperature and photoperiod. (Curves identified in text.)

remained level for several days, pupation was considered complete. The remaining mature larvae were considered to be in diapause.

Field Methods.—Field investigations were conducted in the vicinity of Madison, Wisconsin. Field plantings of corn were used instead of synthetic diets, thereby providing the normal diet and habitat of the borers. To make advanced plants available for very early borer infestation, greenhouse-grown seedlings were transplanted to the field. The plants were transplanted at least 18 days before being subjected to borer infestation. Later plantings were seeded directly in the field. Careful examinations were made of all plants during late June and early July, and any naturally occurring egg masses were removed. A dent variety, U. S. 13 dwarf, was employed in 1959, and a sweet variety, Spancross, in 1960.

Planting dates were replicated four times in a randomized block design. Each plot in 1959 consisted of five plants spaced 10 inches apart, but in 1960 three 2-plant hills 20 inches apart were used. In each case, plots were 80 inches apart, to minimize migration of larvae between plots.

The 1959 experiments were made with borers from a laboratory culture of diverse origin. Newly hatched larvae were reared on the standard purified diet at 30° C. in the dark for 6 days. The larvae were then in the early third instar, and three of them were placed in the whorl of each plant to be infested. This technique provided populations well in advance of natural conditions. However, the failure to expose all instars to field conditions might have had some influence on the incidence of diapause; therefore, in 1960 the plants were infested with egg masses in the blackhead stage. The 1960 borers were the progeny of larvae collected in 1959 from a field in the experimental area. The field from which the population was originally collected was a field of very late sweet corn, and had been infested by second generation borers only.

In 1959, the borers were allowed to remain on the plants until about 725 day-degrees (50° F. base) had accumulated; whereas approximately 960 day-degrees were allowed to accumulate between infestation with eggs and plant dissection in 1960. These temperature accumulations were used to allow enough development to place the majority of the borers in the pupal stage unless they were in diapause. To assure conclusive evidence of diapause, all fifth instar borers recovered from the field were transferred to the laboratory, and held for 2 weeks at 30° C. in the dark in the presence of contact moisture.

RESULTS AND DISCUSSION.—*Incidence of Diapause.*—The results of exposing different European corn borer populations to a series of standardized temperature and photoperiod conditions demonstrated differences among those populations (table 1). If geographical differences in voltinism were explicable as the immediate effects of local conditions on a fundamentally uniform borer population, the several populations tested should have failed to show significant inter-population differences. Mutchmor (1959) attempted to explain the trend of Ontario borer populations toward bivoltinism on just such a basis; he correlated long term changes in voltinism with long term climatic trends. Such an approach failed to consider the evolutionary potential of the borer population itself. The data of table 1 show that borers from different geographical areas differ significantly in their responses to day length and temperature.

Of the populations tested, the incidence of diapause was inversely proportional to both temperature and photoperiod, but the magnitude of response (diapause incidence) was significantly different among the different populations. Under all four of the standard conditions of temperature and photoperiod, the incidence of diapause

was positively correlated with the latitudes from which the populations were obtained. Correlation of response and latitude is indicative of genetic selective pressure exerted by local conditions of day length. However, some populations—most notably those of Kansas and Massachusetts—responded in a manner not consistent with the latitude of their origin, indicating the possibility of evolutionary trends more complex than adaptation to photoperiod alone.

Temperature and Photoperiodic Effects.—Statistical analysis of the effects of 4° C. difference in temperature and 30-minute difference in photoperiod were accomplished by T-test (table 2). In all cases the number of replicates was identical, making the T values comparable in weight.

The data of tables 1 and 2 disclose two separate trends in regard to the effect of small differences in temperature and photoperiod on the incidence of diapause. Some of the populations were much more sensitive to photoperiod than to temperature, within the limited ranges tested. Minnesota, southern Wisconsin, Iowa, Kansas, and Missouri populations showed this characteristic. Missouri borers displayed the greatest response to the 0.5-hour difference in photoperiod. Massachusetts borers were the only population to display an intense effect of temperature on diapause incidence, although significant temperature responses were detected in populations from Minnesota, Kansas, and Missouri. Response differences were not significant for southern Ontario and Pennsylvania populations, although the low incidence of diapause in the controls showed that diapause was induced under photoperiodic influence. It seems probable that the failure to obtain significant differences among the treatments in these populations was attributable to our experimental conditions not falling within the most sensitive range of temperature and photoperiod in all cases. The same explanation might be invoked to account for the lack of significant temperature response differences in southern Wisconsin and Iowa population samples. Nebraska borers yielded a highly significant response to differences in photoperiod, but insufficient numbers of larvae precluded a temperature comparison.

The incidence of diapause under each of the different treatments was shown to be correlated with the latitude from which the population was collected (table 1). The correlation was highly significant from a statistical standpoint, but was not sufficiently strong to allow accurate prediction of diapause incidence on the basis of latitude alone. This may be partially explained as resulting from population differences in temperature response as well as photoperiodic response. Photoperiod is, of course, perfectly correlated with latitude, but temperature accumulation is dependent on other geographical and meteorological factors as well. In addition, natural selection has apparently resulted in diverging population genetic composition during the course of adaptation of the borer populations to differing local conditions. It does not, therefore, appear possible to formulate a means of predicting seasonal occurrence of summer pupation in the absence of detailed information on the temperature and photoperiodic responses of the population in question. This point is well illustrated by comparing the experimental data from Massachusetts borers with those from either Kansas or Missouri. Massachusetts borers have apparently adapted primarily to temperature effects and secondarily to photoperiod. The converse appears to be the case with borers from the more southern states.

Kansas borers reacted to the experimental conditions of temperature and photoperiod in much the same manner as did borers from more northern areas, such as southern Minnesota. When the Kansas borers were first tested, the results seemed so anomalous that all experiments were repeated with a new population sample. The results of the second replicated series were in good agreement with the earlier results, however. The results from testing the Massachusetts population were also strikingly out of line with a strict latitude adaptation. These experiments, too, were repeated with consistent results. In Massachusetts, temperatures during the growing season tend to be much lower than is the case in more western areas of the same latitude. The borer population appears to have evolved a high sensitivity to temperature differences (table 2).

An important population difference was demonstrated in experiments involving borers from southern Ontario and southern Wisconsin. These experiments were designed to resolve an apparently serious discrepancy between the results of Mutchmor & Beckel (1959) and those of Beck & Hanec (1960), concerning the photoperiodic sensitivity of the fifth larval instar. Mutchmor

Table 1.—Incidence of diapause among European corn borer populations under different temperatures and photoperiods.

Population Source	Latitude North	Per Cent Diapause Incidence Under Indicated Temperature and Photoperiod			
		30° C. 14.5 Hr.	26° C. 14.5 Hr.	26° C. 15.0 Hr.	26° C. 0 Hr. (control)
Wausau, Wisconsin	45°00′	100	100	—	54
Southern Minnesota	44°30′	84	98	78	28
Madison, Wisconsin	43°05′	77	90	64	23
St. Thomas, Ontario	42°45′	87	93	89	13
Hadley, Massachusetts	42°30′	2	52	32	0
Central Iowa	42°	66	80	70	17
Lancaster County, Nebraska	40°45′	—	58	21	16
Eastern Pennsylvania	40°30′	40	46	29	0
Jefferson County, Kansas	39°15′	70	96	47	14
Southeast Missouri	36°50′	26	45	4	4
L.S.D. (0.05)		18	5	15	—
Correlation of response and latitude (r)		0.533**	0.579**	0.718**	0.661*

* Significant at the 0.05 level of probability.
** Significant at the 0.01 level of probability.

Table 2.—Statistical analyses (T values) of temperature and photoperiodic effects on the incidence of diapause in different populations of the European corn borer.

Population Source	Temperature Response 26° vs. 30° C.	Photoperiodic Response 14.5 Hr. vs. 15.0 Hr.
Minnesota	2.878*	4.900**
S. Wisconsin	1.000	4.176*
S. Ontario	1.471	2.060
Massachusetts	9.172***	2.259*
Iowa	1.056	3.532*
Nebraska	—	4.894**
Pennsylvania	0.395	0.354
Kansas	2.900*	5.990**
Missouri	2.620*	9.105***

* Significant at the 0.05 level of probability.
** Significant at the 0.01 level of probability.
*** Significant at the 0.005 level of probability.

Table 3.—Growth rates[a] of different populations of European corn borer, reared at 26° C. in the dark.

A Population Source	B Borer-Day-Degrees (50% pupation)	C Coefficient (base = 640)	D Predicted Borer-Day-Degrees in Field (base = 1,450 for first pupation)
Wausau, Wisconsin	540	0.84	1,218
Southern Minnesota	645	1.01	1,465
Madison, Wisconsin	645	1.01	1,465
St. Thomas, Ontario	555	0.87	1,262
Hadley, Massachusetts	480	0.75	1,088
Central Iowa	620	0.97	1,406
Lancaster County, Nebraska	615	0.96	1,393
Eastern Pennsylvania	540	0.84	1,224
Jefferson County, Kansas	655	1.02	1,483
Southeastern Missouri	580	0.91	1,314

[a] Growth to 50% pupation.

and Beckel reported that diapause could be induced by low temperature (18.4° C.) and short photoperiod (9.5 hr. light/24 hr.) in borers that had been reared through the early stadia under nondiapause conditions (high temperature and continuous light). Using somewhat different experimental conditions, Beck and Hanec found that only a small per cent of their experimental borers could be forced into diapause by a transfer from nondiapause conditions (16.5 hr. light/24 hr.) to diapause-inducing conditions (13.0 hr. light/24 hr.) at the beginning of the fifth stadium. In the present study, larvae from both Wisconsin and Ontario populations were started under conditions of 30° C. and continuous light. Larvae that were reared to maturity under such conditions pupated, and no diapause occurred in either population (fig. 1, curve A). When the larvae were 9 days of age (late 4th instar) a group of Wisconsin borers was transferred to 18.5° C. and continuous light; they all pupated within 30 days of age (fig. 1, curve B). Insufficient numbers of larvae prevented a similar treatment of Ontario borers, but Mutchmor & Beckel (1959) had reported similar results. Also at 9 days of age, groups of Wisconsin and Ontario borers were placed under conditions of 18.5° C. and a photoperiod of 9.5 hours of light per day. Of these larvae, 7% of the Ontario group and 25% of the Wisconsin group pupated. The pupation curves (fig. 1, curves C and D) then levelled off. After exposure to this set of conditions for 21 days (30 days of age), the larvae were returned to 30° C. and continuous light (indicated by arrow in figure 1). The larvae were held under these conditions for several weeks. No Ontario borers pupated within a 3-week period, indicating a diapause incidence of 93%. Such results were in good agreement with those of Mutchmor & Beckel (1959). However, a few days later, pupation began, and the pupation curve slowly went to completion. The Wisconsin population showed different characteristics, in that within 5 days after being returned to 30° C. and continuous light, pupation began, and the pupation curve went to completion. The performance of the Wisconsin borers was in agreement with the results reported by Beck & Hanec (1960). The response differences between the two populations appear to account for the

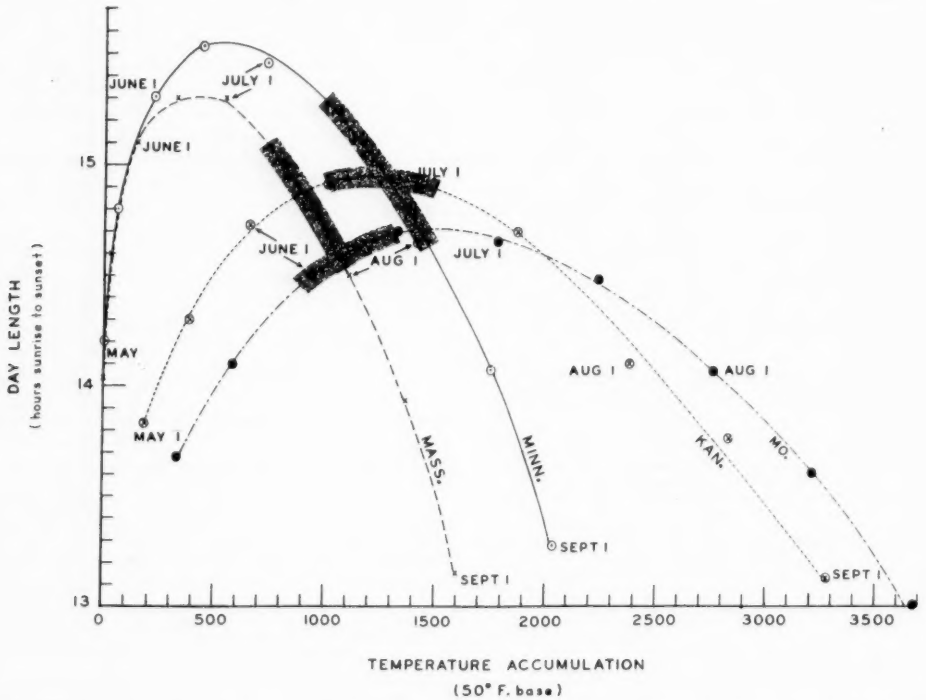

FIG. 2.—Sunrise to sunset day length and normal temperature accumulations in four different geographical areas. The shaded zones on the curves indicate conditions predicted during the last third of the larval developmental period of first generation borers under normal field conditions.

divergence of conclusions drawn by the two research teams. The results also cast some doubt on the criteria used to determine diapause. In effect, diapause was taken as a failure to pupate within a reasonable but necessarily arbitrary time. In the case of the Ontario borers, the levelling off of the pupation curve was taken as diapause; a comparable but shorter levelling off of the pupation curve was not interpreted as diapause in the case of the Wisconsin experiments, although we have considered it an "incipient diapause" of such lability that the larvae may be readily induced to resume development. The Ontario borers displayed an apparently greater stability of incipient diapause than the southern Wisconsin population.

These results imply that field borers, too, may undergo an incipient diapause, during which a period of unusually high temperatures might result in a resumption of development and pupation. Such an effect may account for the early fall moth flight observed in southern Wisconsin in 1960 (Anon. 1960). Based on our experimental results, such a late moth flight would not be likely in southern Ontario.

Temperature and Growth Rates.—In addition to the effects of temperature and photoperiod on the induction of diapause, data were also obtained on the growth rates of borers from the different populations. The borers were reared at 26° C. in continuous darkness, and growth rates were estimated on the basis of the number of days elapsing between hatching and pupation. Since rearing was at a constant temperature, time duration was readily converted to borer-day-degrees, using 50° F. as the developmental base (Apple 1952). Population growth rates were compared on the basis of the borer-day-degrees accumulating from hatching to 50% completion of pupation (table 3).

The temperature accumulation required to attain pupation was found to differ among the several borer populations tested (table 3, column B). Using the average borer-day-degrees required by Iowa, southern Minnesota, and southern Wisconsin borers as an arbitrary "Normal" requirement, 640 borer-day-degrees was taken as the basis of comparing the different populations. Column C of table 3 shows the coefficient of growth for each population. This value was obtained simply by dividing the entry in column B by the base value of 640. Apple (1952) showed that northern Illinois and southern Wisconsin borers required a seasonal temperature accumulation of about 1,450 borer-day-degrees to attain the beginning of pupation under field conditions. Table 3, column D, shows the adjusted borer-day-degrees that might be expected to be required by the populations tested in this study, but under field conditions. This figure was obtained by multiplying 1,450 by the growth rate coefficients entered in column C. The required field temperature accumulations thus predicted are not greatly different from the base of 1,450 in the cases of Minnesota, southern Wisconsin, Iowa, Nebraska, and Kansas. Lower temperature accumulations were indicated for borers from northern Wisconsin, Ontario, Pennsylvania, Missouri, and—especially—Massachusetts. Thus, it is apparent that borer populations in different areas have undergone changes in growth rate as well as in sensitivity to diapause-inducing conditions of temperature and photoperiod.

Table 4.—Normal temperature and day length conditions prevailing during the last third of the developmental period of first generation European corn borer larvae of four different geographical populations.

Population Source	Conditions Predicted During Final Third of Development				
	Temperature Accumulation[a]		Dates		Mean Day Length[b] (hrs.)
	From	To	From	To	
Southern Minnesota	977	1,465	12 July	2 Aug.	14.9
Hadley, Massachusetts	725	1,088	4 July	29 July	15.1
Jefferson County, Kansas	989	1,483	14 June	1 July	14.9
Southeastern Missouri	876	1,314	25 May	14 June	14.6

[a] Borer-day-degrees (F. base)
[b] Sunrise to sunset.

Voltinism.—The implications of these findings in respect to the voltinism manifested by field populations may best be illustrated by considering four different sets of conditions, typified by southern Minnesota, Massachusetts, southeastern Missouri, and Kansas (fig. 2 and table 4). Figure 2 shows the normal seasonal history of day length and temperature accumulation for each of the four areas. Day length was taken as the number of hours from sunrise to sunset, and temperature accumulation was based on normal daily mean temperatures as compiled by the National Weather Records Center. The curves for Minnesota and Massachusetts are similar, except that day lengths are shorter in Massachusetts. The curves for Kansas and Missouri are also similar, but they differ markedly from those of the two northern states. Southeastern Missouri, being south of Jefferson County, Kansas, shows consistently shorter days. Temperature accumulation is normally much more rapid in the two southern states than in either Minnesota or Massachusetts. Missouri temperature accumulation is normally as great by the first of July as that attained 2 months later in Massachusetts.

The developmental characteristics of the European corn borer populations must be superimposed on the physical seasonal history, in order to detect voltinism patterns. Growth experiments, discussed above, show that Massachusetts and Missouri borers required fewer borer-day-degrees to complete their larval development than borers from either Kansas or southern Minnesota. The growth stage at which diapause or pupation is determined is not known with certainty, but it appears to be during the final third of larval development (Mutchmor & Beckel 1959, Beck & Hanec 1960). The conditions of light and temperature prevailing during this critical period are shown in table 4. The critical period in respect to temperature accumulation and day-length is shown for each of the four areas by the stippled sections in figure 2. The critical growth period occurs progressively earlier in the growing season, from north to south. The mean day length prevailing during this period shows no such pattern, however, presumably because of the differential in growth rates among the populations.

Massachusetts borers complete their development under the longest days and lowest temperatures of the four populations under discussion. The low temperature requirement and the relatively low sensitivity to photoperiod apparently results in a low incidence of diapause. This population is the oldest recorded borer population in

North America, and is the classical example of a multivoltine borer. It is well adapted to produce two generations annually under the cool growing conditions prevailing in Massachusetts.

The northern position of Minnesota results in long days but slow temperature accumulation. The Minnesota borer's high temperature requirement and high sensitivity to photoperiod result in a very high incidence of diapause, and an appreciable second generation only in exceptionally early, warm growing seasons. The southern Minnesota population could conceivably evolve toward a higher production of second generation, but such a trend would necessarily involve a speeding up of growth under the existing seasonal temperature accumulation conditions.

The high rate of temperature accumulation in Kansas results in the borers' reaching larval maturity early in the season and under relatively long day conditions. The Kansas borer populations tested displayed a high temperature requirement and a high sensitivity to photoperiod. It seems likely that a high incidence of first generation diapause is prevented only by high mean temperatures. Because of the long growing season, it seems probable that this population will slowly evolve toward a three-generation voltinism.

Missouri temperature accumulation is so rapid that the first generation of borers reaches maturity while day lengths are still increasing. This population also displays a lower than average temperature requirement. These factors combine to produce a very high incidence of summer pupation. It is almost certain that the incidence of a third generation per year will increase rapidly in this population.

An evolutionary trend that might be predicted to increase the differences among geographical populations of the European corn borer will be subject to agricultural modifications. Cultural practices in relation to the time of corn planting will influence the availability of host plants, and thus the establishment of the early first instar larvae. Early infestation of young corn plants will result in high larval mortality because of the presence of the chemical resistance factor 6-methoxybenzoxazolinone (Beck, S. D. 1957). These factors may modify, or even prevent, evolutionary changes otherwise possible.

Prediction of Voltinism in Vicinity of Madison, Wisconsin.—The previous sections have shown European corn borers from various localities to have different responses to temperature and day length under controlled laboratory conditions. The results suggest the necessity of empirical determination of the diapause characteristics of restricted populations in order to predict year-to-year voltinism in relation to day length and seasonal temperature accumulations. This requirement prompted a study during 1959 and 1960 to determine the proportion of borers going into diapause in field infestations made during a 5- to 6-week period during late spring and early summer. The percentage of borers in diapause was calculated on the basis of the forms (pupae and mature larvae) recovered from each planting and infestation (table 5).

The first two groups of larvae in 1959 showed a typical response to long day lengths during June and the first 3 weeks of July, in that all pupated. Thereafter, diapause-inducing conditions prevailed, and the incidence of diapause increased irregularly until the end of the experiment. The borers placed on plants July 30 and removed August 28 were exposed to relatively short days throughout their life on the plants, but only 80% went into diapause. This result agreed with the incidence of diapause (75.6%) among comparable control larvae exposed during the same growth stages to diapause-inducing conditions in the laboratory (26° C. and 12 hr. photoperiod).

The variable results obtained in 1959 and the knowledge gained through measuring the incidence of diapause among other borer populations suggested the use of borers from the vicinity of Madison in subsequent trials. The borers employed in the 1960 experiment came from a local 1959 second generation infestation. This procedure provided a much better indication of the influence of day length and temperature. Although low recoveries from two of the plantings made the observations a little uncertain, there was a pronounced tendency toward an increasing incidence of diapause as the season progressed. These results also indicate that "multivoltine" borers will have but one generation in a year when development occurs under diapause-inducing conditions. It should be pointed out that the results of this field experiment and the results with "Madison" borers in table 1 do not necessarily portray what would be the case with a randomly collected borer population from the vicinity. However, the results show what might be expected from the corn borers that

Table 5.—Incidence of diapause in corn borers reared on field grown corn plants at intervals throughout summer.

Date of Infestation (3rd instar)	Date of Dissection	Number Recovered	Diapause (%)	Date of Infestation (eggs)	Date of Dissection	Number Recovered	Diapause (%)
	1959				*1960*		
June 16	July 14	10	0.0				
23	July 23	4	0.0				
30	Aug. 1	4	50.0	June 14	Aug. 5	52	5.8
July 8	Aug. 10	12	41.7	June 30	Aug. 20	5	20.0
14	Aug. 14	12	66.7	July 8	Aug. 24	8	62.5
21	Aug. 21	28	67.8	July 15	Aug. 31	16	100
30	Aug. 28	15	80.0	July 22	Sept. 6	12	100
Control larvae (under diapause conditions)			75.6				

produce a second generation and constitute the most serious problem to the sweet corn canning industry in south-central Wisconsin.

The information gained in the laboratory phases of this study and in the 1960 field test provide a basis for predicting the degree of summer pupation. Chiang & Hodson (1959) showed that a high degree of pupation at Waseca, Minnesota was associated with years having high temperature accumulation by early July. They suggested that temperatures alone might not be responsible for this relationship, but that day length might also play a role. Their selection of early July as the end-point in temperature accumulation for the prediction of pupation was arbitrary and had no relationship to possible day length interaction. A more precise method for predicting the magnitude of pupation should result from extending the period of temperature accumulation until diapause-inducing photoperiods prevail. Mutchmor (1959) reared borers on synthetic diet in a screened field insectary in Ontario and found no diapause among borers maturing between July 14 and July 19 in 1958, but complete diapause among borers entering the last instar between July 25 and July 30.

The day length under field conditions responsible for diapause cannot be stated exactly because of the interrelationship of temperatures (Beck & Hanec 1960) and the unknown influences of fluctuating field temperatures and of light prior to sunrise and after sunset. During the last week of July at Madison, the hours of sunlight fall below 15 hours per day and the normal mean temperature is 74° F. The results with Madison borers (table 1) showed 64% diapause under conditions of 26° C. (78.8° F.) and a 15-hour photoperiod. Lowering the temperature to 74° F. at this photoperiod would increase the proportion of borers entering diapause (Beck & Hanec 1960). These laboratory findings thus suggest the use of about 15 hours of light at 74° F. to induce a high incidence of diapause. Fifteen hours of light in an incubator must be equal to less than 15 hours between sunrise and sunset, because of the influence of the additional light of twilight on the larvae within the corn plants. Thus, in the following computations, 14.75 hours of light (sunrise to sunset) is assumed to approximate a 15-hour photoperiod in the laboratory.

At Madison, Wisconsin, 14.75 hours of sunlight occurs on July 25. This, then, becomes the critical date after which corn borer larvae have a strong tendency to go into diapause. Mutchmor (1959) found day lengths around July 25 at Chatham, Ontario to be critical in determining the incidence of diapause, but this was without regard to temperature. Temperature accumulations prior to this date play an important part in determining the magnitude of summer pupation. Normal temperature accumulation for July 25 at Madison is 1,354 borer-day-degrees. This is 91 less than the 1,445 borer-day-degrees needed for the first summer pupa, as reported by Apple (1952). One might surmise from this that as a normal condition there is a low order of midsummer pupation at Madison, which field records tend to confirm.

The 1960 data (table 6) might be used to simulate early to late seasons to demonstrate variations in pupation. The first field infestation was made June 14, and the resulting

Table 6.—Theoretical seasonal temperature accumulations for 1960 corn borers in relation to degree of pupation observed.

DATE OF INFESTATION	DATE OF DISSECTION	DAY-DEGREES BY JULY 25	ADDITION OF 1,000 DAY-DEGREES	PUPATION (%)
June 14	Aug. 5	768	1,768	94.2
June 30	Aug. 20	482	1,482	80.0
July 8	Aug. 24	355	1,355	37.5
July 15	Aug. 31	225	1,225	0.0
July 22	Sept. 6	74	1,074	0.0

borers were exposed to 768 day-degrees by July 25. The temperature accumulation from early spring to egg-hatching must be added to this value in order to duplicate the seasonal accumulation. Rather than use the theoretical accumulation up to first egg hatch, it would seem more realistic to use the 1,000 day-degrees suggested by Apple (1952) for the occurrence of 50% hatch. The borers involved in the June 14 infestation thus had the equivalent of 1,768 day-degrees before July 25, with an accompanying high rate of pupation (94.2%). This condition is typical of a very advanced season for Madison. Similar computations were made for the other infestation groups (table 6, fig. 3). The last two groups of larvae developed under very retarded temperature and day length conditions, and no individuals were able to pupate. Because of the variation in adaptation to photoperiod and temperature, the predictive value of figure 3 is applicable only to the vicinity of Madison, Wisconsin.

The present study can be considered no more than a preliminary demonstration of the existence of marked differences in the developmental characteristics of different geographical populations of the European corn borer. It has been shown that the terms "univoltine" and "multivoltine" are with little meaning in the description of borer populations. In view of the practical implications and ecological importance associated with the population differences demonstrated, a cooperative regional study of corn borer populations should be initiated. Such a project should undertake the characterization of borer populations and the determination of the population trends that may determine the characteristics of the European corn borer in the future.

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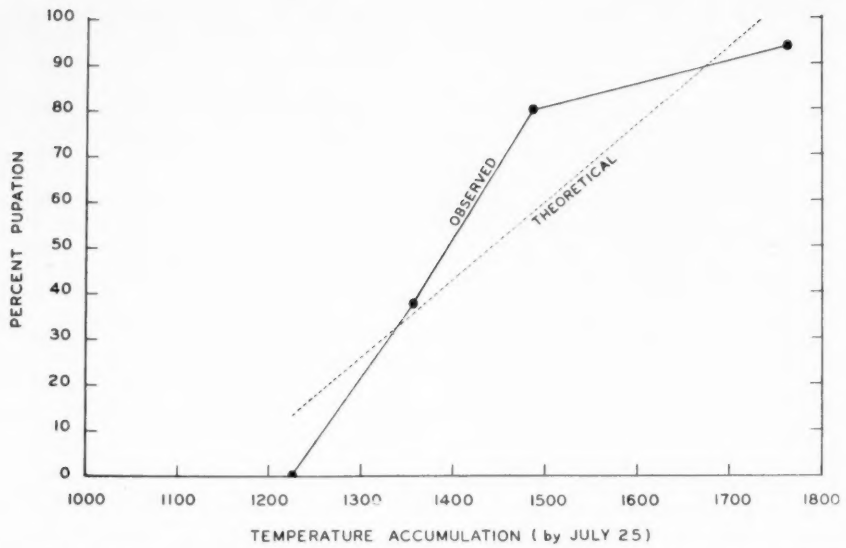

FIG. 3.—Seasonal temperature accumulations by July 25 and the incidence of midsummer pupation among European corn borer larvae in the vicinity of Madison, Wisconsin.

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The Navel Orangeworm in Northern California[1]

A. E. Michelbacher and Clarence S. Davis,[2] *University of California, Berkeley*

Abstract

The navel orangeworm, *Paramyelois transitella* (Walker), which was first found in California in 1942 has developed into a serious pest of walnuts. The insect is a scavenger, but seriously attacks sound walnuts once the husks crack as maturity is reached. There are a number of generations each year. The insect has no diapause and passes the winter as a caterpillar.

The navel orangeworm carries over from one crop to the other in walnuts on the ground or in the trees. By early summer these breeding sources are all but eliminated. At this period walnuts of the current season's crop infested with the codling moth furnish excellent breeding sites. They fill the developing gap in the food chain. Although less important than the codling moth, walnut blight, *Bacterium juglandis* Pierce, adds to the problem as do other factors responsible for the production of unsound walnuts.

Heat and dryness are unfavorable for optimum development of the navel orangeworm.

An effective spray program against the pest has not been developed. However, the following practices are useful in holding a potentially destructive population below a serious economic level: Secure excellent control of the codling moth; discourage walnut blight; harvest at the earliest possible date; immediately dry the crop; fumigate crop as soon as dried; keep storage areas dry; practice orchard and plant sanitation.

The navel orangeworm, *Paramyelois transitella* (Walker), has developed into a serious pest of walnut in northern California. The insect is primarily a scavenger, but is capable of attacking sound walnuts after the husks have cracked.

It was first recorded for California in 1942 where it was encountered breeding in mummified fruits in Orange County (Keifer 1947). By 1946, it became a threat to the walnut industry in the southern portion of the State. The fullest account of the insect activity on walnuts in southern California is that given by Ortega (1950). He reported that the infestation reached maximum proportions in 1947.

The navel orangeworm did not become established in northern California until sometime after 1946. It first showed evidence of being seriously destructive to almonds (Bacon & Wade 1954). The pest spread into all the major walnut growing sections and by 1954 damaging infestations were encountered (Lockwood 1954, Michelbacher & Ross 1955). Except for minor fluctuations, the navel orangeworm problem has continued to increase. A most serious potential confronted walnut growers in 1959 and 1960. Observations were made that indicated that potentially destructive populations are able to maintain themselves in walnut groves throughout the year. Infestations in walnuts may be augmented by migrations from other sources but destructive populations in walnuts are not dependent upon such activity. It appears that the greatest threat from the insect arises from conditions that exist within the orchards.

The navel orangeworm has no diapause. Breeding continues until stopped by cold weather. During the late fall and winter, larvae in the nuts continue slow development. By late winter and early spring development is completed, mating occurs and a new generation is started. From spring through fall there are a number of overlapping broods. The larvae spin considerable webbing and from one to more than 25 individuals may develop in a single nut. Usually adults are not encountered in the field although under conditions of heavy infestations they may be seen frequently at harvest time.

Role of Codling Moth.—The codling moth (*Carpocapsa pomonella* (L.)) plays an outstanding role in the ecology of the navel orangeworm. Because of its scavenger habits, nuts infested by the codling moth furnish ideal breeding places. A rise in the navel orangeworm population is associated with an increase in the codling moth infestation (Ortega 1950, Michelbacher & Ross 1955, 1957, Michelbacher 1956). The correlation that exists between the two insects is most marked.

Nuts infested by the codling moth probably constitute the most important breeding source that carries the navel orangeworm over from one crop to the other. The reason for this is that the navel orangeworm is unable to penetrate the husks of sound nuts. Such nuts are not subject to attack until the husks begin to crack with the approach of maturity. The population of the navel orangeworm usually reaches its lowest level in early summer. By that time breeding sources from the old crop are nearly dissipated. The food chain that carries the insect over from one crop to the next is all but broken. It is at this time that walnuts infested by the codling moth fill in the gap. These infested nuts are excellent for breeding and, if a sizeable codling moth infestation prevails, breeding continues in them and by harvest time a large population of the adult navel orangeworm may be present to attack sound nuts as the husks split.

In table 1 is shown the relation that existed at harvest between the codling moth infestation and that of the navel orangeworm in the 1960 experimental plots at Linden, California. The data clearly show that the number of nuts attacked by the navel orangeworm increased with a rise in the codling moth infestation. This is typical of what has occurred in all experimental studies where the two insects have been involved.

Role of Walnut Blight.—Although probably of much less importance than the codling moth, walnut blight, *Bacterium juglandis* Pierce, is a factor in the ecology of the navel orangeworm. Developing walnuts are seriously infected by this disease. Early evidence indicated that infected nuts were not a suitable breeding source for the navel orangeworm. This conclusion was reached because of the inability to rear out specimens

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Table 1.—Per cent of infested walnuts in harvested crop in the 1960 experimental plots at Linden, California.

Insecticide and Pounds per Acre	Date Applied	Per Cent Infested Nuts		
		Codling Moth	Navel Orangeworm	
			Primary	Secondary[a]
Check	—	14.0	3.0	3.7
Sevin®,[b] 50% W.P., 10	April 21 June 20	1.70	0.70	0.65
DDT, 50% W.P., 12	April 20	0.55	0.63	0.15
Guthion®,[c] 25%, W.P., 6	April 22 June 21	0.32	0.48	0.10

[a] Navel orangeworm infesting nuts previously attacked by codling moth.
[b] 1-naphthyl *N*-methylcarbamate.
[c] *O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate.

from totally destroyed nuts collected during the summer. However, later observations showed that in the spring blighted nuts that remained in the trees did serve as a very poor breeding source. On rather rare occasions such nuts could be found infested, but usually the caterpillar lacked vigor.

In the 1959 season many nuts were infected with walnut blight where the disease was arrested before the nut was completely destroyed. Often the husk and shell at the point of lesion would crack exposing the meat chamber. These nuts and those similarly infected served as suitable breeding places. Such nuts, like those infested with the codling moth, served to bridge the gap between the old and new crop. Breeding in these nuts expanded the population and added to the likelihood of sound nuts being attacked after the husks cracked. Conditions similar to 1959 were encountered again in 1960. Observations certainly showed that walnuts infected with arrested walnut blight can create a serious menace to the new crop.

Role of Other Unsound Walnuts.—Due to a variety of factors other than codling moth and walnut blight, there are usually a number of walnuts where the meats fail to develop normally. These nuts may be blanks or they may contain shriveled or otherwise injured meats. Where no meats are found, the nuts are not likely to be infested with the navel orangeworm. Where meats are present in various stages of development, navel orangeworm activity can be expected. The husks on these unsound walnuts tend to crack prematurely and are thus subject to attack by the navel orangeworm a month to several weeks before sound nuts are in a receptive stage. At harvest the culls are frequently heavily infested but are seldom a menace to sound nuts where early harvesting practices are followed. However, they do constitute a serious hazard where harvest is delayed until after the adults begin to emerge, and in the gleanings.

Role of Temperature and Moisture.—Temperature and moisture are very important in the activity of the navel orangeworm. Wade (1958) noted that the number of instars increased as the temperature increased or decreased from 27.5° C., or as the relative humidity decreased from 95%. From this observation it is apparent that the navel orangeworm does best at a moderately high temperature and under rather moist conditions. At harvest both temperature and moisture content of the nuts are favorable for the activity of the pest. However, as the nuts dry there is evidence that they are less subject to attack. Also, where they are exposed on the ground to direct rays of the sun there is the possibility of the temperature rising to a lethal level, particularly in the case of small caterpillars. The nature of the soil also appears to exert some influence. Apparently a sandy soil will heat to a higher level than a loam. Soil moisture conditions influence the temperature as does the soil cover. Nuts in protected and moist locations are likely to be more heavily infested than those in open dry areas. In both 1959 and 1960 the potential of the navel orangeworm as a pest prior to harvest appeared to be about equal. However, in 1959 heavy rains occurred, while in 1960 dry conditions existed during and following harvest. Nuts left unharvested on the ground showed a considerably higher degree of infestation in 1959 than in 1960. The infestation of the nuts remaining in the trees was more nearly the same.

Dryness of the nuts may be one explanation for the navel orangeworm not breeding in storage to the same extent it does out-of-doors. It may be one of the reasons that it is considered more of a field pest than a storage pest, although under favorable conditions troublesome populations can develop in storage.

As fall advances, temperatures drop to a point where adult activity comes to an end and further infestation of nuts in the field ceases.

Although caterpillars are able to survive the winter, further information on this subject is needed. Evidence so far accumulated indicates that larvae in the early instars are probably less resistant to cold than are those that are more mature. Such a situation might be of considerable importance in a severe winter. The temperatures of the past two winters (1958–60) have been mild. Little winter mortality from physical factors has been detected. This fact could have had an important influence in maintaining the insect at a potentially dangerous level. It would be most desirable to have an opportunity to study the survival ability of the insect during a severe winter.

The past 2 years (1959 and 1960) have been dry as well as mild. Under the dry conditions unharvested nuts in the field have not deteriorated as rapidly as they would in a wet year. This could be an important factor in maintaining the pest at a threatening level.

Importance of Early Harvest.—Because the navel orangeworm is unable to enter sound nuts until the husks split, every effort should be made to harvest the crop at the earliest possible date. Where there is a delay an increase in the navel orangeworm infestation can be expected and this can be of serious proportions. Prompt harvest can reduce the infestation to an exceedingly low level even in those cases where a potentially destructive population is developing in the nonmerchantable portion of the crop. It is possible to harvest the sound nuts before the pest has had an opportunity to infest them. A relatively small loss has been encountered in the experimental orchard at Linden where this practice has been followed. Where there is a delay in harvest the sound nuts are heavily attacked.

The navel orangeworm gains entry into sound nuts at the stem end. The closure may not be complete and for this reason the caterpillars usually enter the nuts without leaving any external evidence of their entry. As a result it

Table 2.—Seasonal trend of infestation of the navel orangeworm in unharvested walnuts on the ground and in the trees, and the number of clerid larvae per 100 nuts.

	Walnuts from Ground				Walnuts from Trees			
		Infested Nuts with Living Individuals[a]				Infested Nuts with Living Individuals[a]		
Date (1959–60)	Number	Per Cent	Average Number per Infested Nut	Clerid Larvae[b]	Number	Per Cent	Average Number per Infested Nut	Clerid Larvae[b]
Nov. 25	200	18.0	1.8	0.0	200	20.0	3.4	0.0
Dec. 18	200	16.5	2.0	0.0	200	25.0	3.0	0.0
Feb. 16	200	35.0	2.8	1.5	52	32.7	3.0	0.0
March 16	200	33.5	3.0	0.5	100	39.0	4.3	1.0
March 29	—	—	—	—	100	54.0	6.1	11.0
April 5	200	10.0	1.5	0.0	100	36.0	4.2	0.0
June 2	335	1.7	1.7	14.5	—	—	—	—
June 13	122	4.9	1.8	8.2	—	—	—	—
June 21	—	—	—	—	100	24.0	7.4	17.0

[a] Includes larvae and pupae.
[b] *Cymatodera oripennis* LeC.

is almost impossible to sort out infested nuts and the presence of infestation can usually be detected only by cracking the nuts. However, as the caterpillars approach maturity they tend to eat through the soft tissue at the stem end or even directly through the hard shell. The caterpillars have strong jaws and have a rather remarkable power of penetration. In the late stages of development exit holes and associated webbing give evidence of infestation. Pupation occurs within or outside of the infested nut.

IMPORTANCE OF RAPID DRYING OF HARVESTED CROP.—As soon as the nuts are picked up they should be thoroughly dried. Dried nut meats are less favorable for infestation and development than moist meats. It is certain that the rate of development is markedly retarded by rapid drying. In the case of first instar larvae, growth may be so reduced that the nuts can be delivered to the packing house and fumigated before injury progresses to a point where it can be detected in a crack test of the nuts.

IMPORTANCE OF SANITATION.—The principal source for carrying over the navel orangeworm from one crop to the next is the crop refuse left in the field. The pest could be very well controlled if this material were completely destroyed before the new crop is subjected to attack. Following harvest breeding material declines and usually is all but eliminated before the new crop is receptive.

The most important breeding source is the sound nuts that remain on the ground and in the trees. By early summer, winter rains, cultivation, and irrigation take a heavy toll of the nuts on the ground. Where good farm practices are followed, most of these are destroyed, although under conditions of unusual dryness a considerable number may remain until nuts of the new crop are available. Nuts remaining in the tree are of greatest importance in carrying the insect over to the new crop. During the fall, winter, and spring these nuts continue to drop, but usually enough remain in the trees to carry the insect over. The complete destruction of these remaining nuts presents a most difficult problem.

In the experimental orchard at Linden, samples of nuts were collected from the ground and from the trees from November 1959 into June 1960. As the season advanced it became more difficult to find nuts. After June 13 cultivation and irrigation completely eliminated nuts on the ground. Prior to this time decay and weathering all but rendered the available nuts unsuitable for breeding. The last collection from the trees was made on June 21 when a great deal of searching was required to find any nuts. The results obtained are given in table 2. Although navel orangeworms were found in all collections, it is readily apparent that nuts remaining in the trees exceeded those on the ground in carrying over the pest to the new crop. In March there was an increase in the number of individuals encountered, probably marking the start of reproduction for the new year.

The number of navel orangeworms encountered in nuts on the ground will vary according to cultural practices and moisture conditions in the orchard. It can be more or less than that encountered in the experimental orchard at Linden. For example, less cultivation and dryer conditions existed in an experimental orchard at Walnut Creek. On June 10, $7\frac{1}{2}\%$ of the walnuts on the ground were infested and the average number of individuals per infested nut was 3.2 which was considerably higher than the figures obtained at Linden for June 13.

In addition to orchard sanitation, a cleanup of possible breeding sources around hullers and packing sheds is desirable. Culls and all other refuse should be destroyed.

SIZE OF ORCHARDS.—In general the navel orangeworm has presented a more serious problem in the smaller orchards. Such orchards are more influenced by adjacent planting than are large ones. Also, the navel orangeworm is frequently most troublesome about the homestead. This is probably due to the fact that more hosts are usually present, and an effective sanitation program is more difficult to operate. It is well to keep in mind that most mummified fruits and nuts such as almond are hosts of the navel orangeworm.

NATURAL ENEMIES.—It is difficult at this time to ascertain just how important natural enemies will become in checking damage by the navel orangeworm. Ortega (1950) reared the parasitic wasp, *Microbracon hebetor* (Say), from larvae. Wade (1956) observed the parasitic wasps, *Perisierola breviceps* Krombein and *Mesostanus gracilis* Cr., attacking the pest. He also reported the black hunter

thrips, *Leptothrips mali* (Fitch), as being a possible predator on the eggs of the navel orangeworm. In the present study the larvae of the clerid, *Cymatodera ovipennis* LeC., has been encountered on a number of occasions attacking the larvae of the navel orangeworm. This predator is a natural enemy of caterpillars. An account of this insect is given by MacSwain (1945). The predator is rather widely spread in central California, and exhibits some promise against the navel orangeworm. The frequency with which it was taken in the experimental orchard at Linden is given in table 2. In late spring and early summer the larvae were encountered in fair abundance and were sufficiently numerous to indicate that at this time of the year they are probably of some importance in destroying navel orangeworm caterpillars. In 1960 specimens of the predator were taken in nut samples collected as early as October 21.

The common insect fungus, *Beauveria bassiana*,[3] has also been found attacking larvae of the navel orangeworm.

CONTROL.—No effective spray program against the navel orangeworm has been perfected. Control is dependent upon orchard and plant management. There are a number of practices that can be followed that will hold the navel orangeworm population below a serious economic level. This is possible even where a potentially dangerous population exists. For reasons discussed above, the following practices are encouraged: Where the codling moth presents a problem use an effective spray program to control it; follow practices that discourage walnut blight; harvest and dry crop at the earliest possible date; fumigate crop as soon as dried; keep storage areas dry; practice orchard and plant sanitation, with particular emphasis on destruction of crop refuse before the new crop is subject to infestation.

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[3] Identified by Dr. Edward A. Steinhaus.

Tests Against Face Flies on Cattle in New Jersey During 1960[1]

PHILIP GRANETT and ELTON J. HANSENS, *Department of Entomology, Rutgers—The State University, New Brunswick, N. J.*

ABSTRACT

In tests with formulations of repellents, insecticides, insecticides plus repellent, and insecticides plus attractant, applied as wipes, paints, or sprays against face fly (*Musca autumnalis* DeGeer) on dairy cattle in New Jersey, no treatment was considered outstanding from the viewpoint of reducing annoyance from this pest. A few hours of relief was attributed to the use of synergized pyrethrins plus repellents such as Crag Fly Repellent® (butoxy polypropylene glycol) or MGK R1207 (3-chloropropyl *n*-octyl sulfoxide) applied as a face wipe or as a spray directed at the head. Dilution of a methoxychlor-Crag Fly Repellent concentrate to 0.5% methoxychlor and 5% Crag Fly Repellent appeared to have slightly better residual action but it would need to be applied daily. Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) (at 0.2% to 0.5% in a water dispersed spray) applied daily to faces of cattle appeared to result in a moderate reduction of face flies. Although no untoward effects were noted in these tests, continued treatment with Dibrom would require that a formulation be developed which would not be irritating to animals or operator. Other toxicants which were tried and found to be of doubtful value as used were CO-Ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate), Dylox® (dimethyl(2,2,2-trichloro-1-hydroxyethyl) phosphonate), Diazinon® (*O,O*-diethyl *O*-(2-isopropyl 6-methyl-4-pyrimidyl) thiophosphate), GC4072 (diethyl-1-(2,4 dichlorophenyl)-2-chlorovinyl phosphate), dimethoate, and DDVP. Combinations of these with attractants such as corn syrup or granulated sugar spread on the faces of the animals also failed to provide relief from annoyance. Continued use of these baits caused the hair on the cows' head to become matted and sticky. As a favorable by-product of frequent treatment with formulations containing insecticides, annoyance from horn flies (*Haematobia irritans* (L.)) and stable flies (*Stomoxys calcitrans* (L.)) on the animals' bodies was less than is usually observed in the summer season.

The extent of annoyance to cattle (and horses) by face flies (*Musca autumnalis* DeGeer) has increased rapidly in New Jersey during the past 2 years. As many as 50 flies per head is not uncommon; even a relatively few flies,

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however, appear to disturb and irritate the animals because of the fly's habit of feeding on secretions of eyes and nose. This problem is, of course, not confined to New Jersey; a review of the life history and habits of this pest (Teskey 1960) indicates that face flies are found now in abundance in many areas of eastern North America.

In preliminary tests in New Jersey last year it was found that use of conventional fly control materials and procedures were only moderately successful in reducing face fly annoyance. On the other hand, Bruce *et al.* (1960) reported good control with insecticide baits painted on the faces of the animals, and Treece (1960) and others have stated that synergized pyrethrins may offer some relief.

CONDITIONS OF THE TESTS.—In the current work, measures which appeared to have even some modicum of success elsewhere were considered for trial under New Jersey conditions. Eventually various formulations containing repellents, insecticides, combinations of an insecticide with a repellent, or combinations of an insecticide with an attractant to form a toxicant bait were tried. These chemicals were applied to the animal's head in various ways. One method was a *wipe*, in which approximately 5 ml. of the formulation was spread on the face with a piece of cheesecloth wet with the substance being tried. A variation of this was the use of a 1-inch brush to *paint* approximately 3 grams of the material between the eyes of the treated animal. A thick syrup formulation contained in a flexible plastic bottle also was *streaked* on the face of the animal directly from the container. Application was also made by means of water- or petroleum-oil-based *sprays;* a knapsack or small electric sprayer was used for this purpose. One ounce of oil and 2 ounces of the water-carried sprays were usually applied to the face and neck of each cow.

At least six cows (mostly Holsteins) were used in each test, and the application of each formulation was made in the morning immediately after milking. With few exceptions the tests were replicated several times. Counts of flies on the animals' faces were made in the pasture or feed lot on the day of application and, whenever possible, on the next day.

As in other types of field evaluations, apparent variations in results might be influenced by changes in the activity of flies brought about by such changing conditions as weather, movement (or lack of it) of the cattle, the numbers of flies in the locality, the time of day when observations were made (which might vary to suit the convenience of the observer), and the individual differences of the animals. The degree of effectiveness was usually judged on the basis of the reduction in numbers of flies on treated cattle as compared with the numbers on untreated animals in the same herd. Because some protection is sometimes evident on untreated animals in a treated herd, especially when insecticide-repellent formulations are used, this method of measuring effectiveness may result in an apparently lower level of control than when counts of flies prior to treatment are used for comparison or if a treated herd is compared with a nearby untreated herd. On the other hand, even if prior counts or another herd are available, the various factors influencing the number of flies on cattle may differ from the conditions which may exist when the treated herd is examined. Despite these variables, a pattern usually develops from which general conclusion can be made on the relative degree of effectiveness of the formulations tried, although direct comparison between treatments made at different times and periods of the season may not be justified.

REPELLENTS.—A summary of the results obtained with three repellents alone or in combination with each other, formulated in Bayol D, a refined petroleum oil, is shown in table 1,A. From 50% to 70% protection was obtained from Crag Fly Repellent® (butoxy polypropylene glycol) at 5% to 20% concentrations, and MGK R1207 (3-chloropropyl *n*-octyl sulfoxide) at 2% for a few hours after application as a wipe although most of this was lost by the following day. Combining the repellents with MGK 264 (N(2-ethylhexyl)-bicyclo[2.2.1]-hept-5-ene-2,3-dicarboximide) as a synergist did not appear to influence repellency markedly. Tabatrex® (dibutyl succinate) as a spray was not effective 6 hours after application, when the cows were observed.

INSECTICIDES.—The results of tests with nine chemicals are shown in table 1,B.

Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) as a 0.2% to 0.5% water spray provided moderate to good reduction for 6 to 8 hours after application, but relatively little protection was evident from it the following day. A more limited test with Dibrom in oil did not appear to be as effective. Unfortunately, although no untoward effects were noticed during its use, these formulations of Dibrom may, on repeated use, cause irritation to the animals or the operator. The synergized pyrethrin sprays reduced fly annoyance for 1 to 4 hours after application but thereafter effectiveness was poor. The addition of 0.75% methoxychlor, in a formulation containing a lower synergized pyrethrin content did not appear to alter the results materially.

Diazinon® (*O,O*-diethyl *O*-(2-isopropyl 6-methyl-4-pyrimidyl) thiophosphate) and GC4072 (diethyl-1-(2,4 dichlorophenyl)-2-chlorovinyl phosphate) both used at 0.5% in water, were effective for a few hours but thereafter flies on the animals were again numerous.

Dimethoate at 1%, Dylox® (dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate) at 2%, Co-Ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate) at 0.5% in water, and Sevin® (1-naphthyl *N*-methylcarbamate) at 1% plus piperonyl butoxide in oil were relatively ineffective. Not shown in the summary table, Silikil dust (silica gel containing ammonium fluosilicate), Silikil plus 0.05 synergized pyrethrins, and Silikil plus 0.5% DDVP applied as dusts to the face of the animal provided no reduction in flies over the period of the test.

INSECTICIDE PLUS REPELLENT.—Combinations of nine insecticides with Crag Fly Repellent were tested to determine whether repellent action could be augmented. The results of these tests are summarized in table 1,C.

Used as a wipe or as a spray a 1 to 9 dilution in Bayol D of a methoxychlor-Crag Fly Repellent concentrate (5% methoxychlor, 50% Crag Fly Repellent, of the Union Carbide Chemicals Company) was moderately effective (55% to 70% repellency) on the day applied and on the following day afforded about 50% protection. An applica-

Table 1.—Effect of various formulations applied to cattle to reduce face fly annoyance.[a]

Treatments and Type of Application	Active Ingredient (%)	No. Applications	Total No. Animals	Average Number of Flies per Animal and Per Cent Repellency							
				1–2 Hrs.		4–5 Hrs.		6–8 Hrs.		1 Day	
				No.	%	No.	%	No.	%	No.	%
A. Repellents in Bayol D											
Crag Fly Repellent											
Wipe	5	1	6	4	51	5	50	8	40		
Spray	10	1	6					18	35		
Wipe	20	1	6	4	69	12	39	9	53		
MGK R1207											
Wipe	2	2	12	5	69	4	75	7	15	15	12
Spray	2	1	6					21	19		
MGK R1207	2										
+Crag F.R.	5										
+MGK 264	2										
Wipe		2	12	7	62	5	72	4	50	24	13
MGK R1207	2										
+MGK 264	5										
Wipe		1	6	6	69	12	39	9	53		
Tabatrex											
Spray	5	1	6					25	6		
B. Insecticides											
Co-Ral W.P.											
Water spray	0.5	2	26	12	2			13	0		
Diazinon E.C.											
Water spray	0.5	3	18	5	44	8	47	8	42	9	28
Dibrom E.C.											
Water spray	0.25–0.5	8	256	2	76			3	69	6	38
Oil	0.5	1	12					18	23	20	11
Dimethoate E.C.											
Water spray	1	3	18	5	25			4	0	12	0
Smear	1	3	18	7	45			6	0	2	43
Smear	2	3	18	8	35			11	0	7	0
Dylox W.P.											
Water spray	2	3	18	6	51			3	53	10	25
GC 4072 E.C.											
Water spray	0.5	3	18	6	56	9	42	6	26	13	24
Pyrethrins	.25										
+piperonyl butoxide	.2										
oil spray[b]		7	70	4	62			5	53		
Pyrethrins	.1										
+piperonyl butoxide	.12										
+methoxychlor	.75										
oil spray[c]		4	44	3	66			7	35		
Sevin	1										
+piperonyl butoxide	1										
oil spray		2	12	16	46			23	0	5	12
C. Insecticides and Repellents Combined											
Diazinon E.C.	0.5										
+Crag F.R.	5.0										
Water spray		3	18	4	49	7	56	9	37	9	23
Dimethoate E.C.	1.0										
+Crag F.R.	5.										
Water spray		3	18	4	47			4	49	5	26

[a] Per cent reduction based on comparison with counts made on untreated animals in the same herd.
[b] Gulf Spray Regular.
[c] Gulf Spray Special.

Table 1.—(*Continued*)

Treatments and Type of Application	Active Ingredient (%)	No. Applications	Total No. Animals	Average Number of Flies per Animal and Per Cent Repellency							
				1–2 Hrs.		4–5 Hrs.		6–8 Hrs.		1 Day	
				No.	%	No.	%	No.	%	No.	%
Dylox	2.0										
+Crag F.R.	5.0										
Water spray		3	18	6	60			3	41	9	11
Malathion E.C.	0.5										
+Crag F.R.	5.										
Wipe		1	6	2	58			8	50		
Methoxychlor	0.5										
+Crag F.R.	5.0										
Oil wipe		3	18	2	69	2	70	4	55	5	59
Oil wipe[d]		2	12	11	64	15	55			4	52
Methoxychlor	1.0										
+Crag F.R.	10.0										
Oil wipe[d]		1	6	3	52	6	52	7	56		
Pyrethrins	0.025										
+piperonyl butoxide	0.2										
+Crag F.R.	5.0										
Spray[e]		3	18	5	60	7	54	10	23	14	41
Pyrethrins	0.12										
+piperonyl butoxide	.24										
264	.4										
Crag F.R.	10										
Oil spray[f]		8	76	4	74	5	61	6	57	17	32
GC 4072	0.5										
+Crag F.R.	5										
Water spray		3	18	4	67	7	42	7	22	10	23
Ronnell	5										
Pine oil	35										
Wipe		1	6	2	77	3	61	3	50	5	35
D. Insecticides and Attractants Combined											
Diazinon	0.5	3	18	3	55	6	40	13	18	12	11
+Sugar water spray											
DDVP	0.5										
+Syrup ("Banafly")[f]											
Wipe		2	12	5	20	3	30	4	21	12	9
Streak		4	52			6	37	12	16		
DDVP	0.2										
+Syrup											
Painted		7	126					20	10		
Dimethoate	0.5										
+Sugar		3	18	8	3	4	0			13	0
Water spray											
Dimethoate[g]	1.0										
+Syrup											
Wipe		3	18	12	0	17	0			8	0
Dimethoate[g]	2.0										
+Syrup											
Wipe		3	18	6	10	7	0			5	0
Dylox	2.0										
+Sugar											
Water spray		3	18	8	29			4	19	12	18

[d] From "Sovachlor" or F-21 concentrate, Union Carbide Chemical Co.
[e] From Fairfield Chemicals Co.
[f] From "Banafly" spray concentrate, American Scientific Lab., Inc.
[g] Formulations from American Cyanamid Co.

tion in which the concentration of the active ingredients was doubled did not increase effectiveness. The use of Crag Fly Repellent with synergized pyrethrins, Diazinon, dimethoate, Dylox, malathion, and GC4072 appeared to boost, at least temporarily, the effectiveness of these insecticides. The ronnel smear was placed in this group because of its pine oil content. It, too, appeared to be effective only on day of application.

INSECTICIDES PLUS ATTRACTANT.—The attractant used was either corn syrup (usually 2 parts Karo syrup to 1 part water) or granulated sugar at the rate of 25 pounds per 100 gallons water. The results when these were combined with four toxicants and tried against face fly are shown in table 1,D.

Although to these flies the baits were undoubtedly attractive and toxic in many instances, despite the treatment face flies continued to feed on secretions around the eyes and nose of the animals; the bait was not attractive enough to draw flies away from these natural feeding sites.

DDVP, at 0.5% (in the Banafly product) and at 0.2% in Karo syrup, failed to cause any marked degree of reduction of flies in several trials of 3 or 4 days daily application. Because rain interrupted the test schedule, it was not possible to extend the period for a 2-week period as suggested in label recommendations for the DDVP-syrup bait. From the observations made, however, it is questionable whether the farmer would adhere to a schedule of daily applications if he continued to see the animals plagued with flies. To be effective in reducing fly populations with such applications it would also be necessary to convince farmers with neighboring herds to adopt similar control procedures.

Daily treatments also caused the hair, where the bait was applied, to become matted and sticky. This in itself may be a source of annoyance to the animal and herdsman.

The dimethoate baits were applied over a period of several weeks, in some cases every other day and in other instances for 3 daily applications. Although fly numbers appeared to be reduced slightly, in no instance was reduction evident 24 hours following the last treatment.

Neither Dylox nor Diazinon combined with sugar were any more effective in the limited tests with these substances than when the toxicants were used without the bait.

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The Effect of Sevin on Honey Bees[1]

ROGER A. MORSE, *Cornell University, Ithaca, New York*

ABSTRACT

Aerial applications of 1¼ pounds of Sevin® (1-naphthyl *N*-methylcarbamate) per acre were made on 73,610 acres in two counties in New York State between May 21 and June 10, 1959. Twenty-one colonies of honey bees were placed in five locations within the treated area. Five check colonies were placed 3¼ miles outside of the treated area. Gary traps were placed on all colonies. During the spray and postspray period of 47 days treated colonies lost a mean of 19,917 bees while check colonies lost a mean of 2,936 bees. Mortalities were above normal for up to 3 weeks following the insecticide applications. Colony recovery was rapid. Some cases of European foulbrood resulted from weakening of the colonies.

In December of 1958 the Plant Pest Control Division of the United States Department of Agriculture announced that approximately 75,000 acres of land in Otsego and Delaware Counties in New York State would be aerially sprayed with Sevin® (1-naphthyl *N*-methylcarbamate). The pest insect was the gypsy moth (*Porthetria dispar* (L.)) and the application rate was 1¼ pounds per acre.

As a result of this announcement an experiment concerning the toxicity of Sevin to honey bees (*Apis mellifera* L.) was undertaken in North Carolina in cooperation with the Plant Pest Control Division and North Carolina State College.[2] On April 5 between 7:05 and 7:18 a.m. 24 acres of land on which 24 colonies of honey bees each occupying 1½, 2, 2½, or 3 supers were located was sprayed at the rate of 1.08 pounds of technical Sevin per acre. Temperatures taken at the Raleigh airport, some 20 miles distant, showed that the maximum temperature for the day was 73 and the minimum 36 degrees F. There were very few nectar plants in bloom in the sprayed area.

An examination of the colonies, which were placed randomly in five locations in a lightly wooded area, both before and immediately after the spray operations showed no visible effects. By 10:30 a.m. there was considerable activity at the colony entrances and the bees were gathering water from several pools in a roadway running through the woods. Droplets of oil and insecticide were visible on the surface of the pools. The heaviest flight occurred between 11 a.m. and 4 p.m. Professor W. A. Stephen examined the colonies on April 11th and reported that no abnormal conditions were apparent. As a result of these experiments the Plant Pest Control Division was advised to expect no serious bee losses under similar circumstances.

Between May 21 and June 10, 1959, 73,610 acres in

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Table 1.—Relation of applications of Sevin to death of bees and honey production.

Apiary	Number of Applications in Apiary Vicinity	Distance from Apiary (miles)		Gallons of Spray		Dead Bees Recovered per Colony	Mean Gain in Weight (lb.)	
		Range	Mean	Total	Mean		6/12–8/14	6/12–10/9
3	6	0–0.85	0.37	11,234	1,872	31,691	9	26
4	4	0–0.7	.47	9,607	2,401	25,283	39	108
5	10	0–1.2	.60	15,207	1,521	22,930	30	78
2	9	0–1.0	.60	11,838	1,315	12,299	46	113
1	6	0–1.9	.75	8,723	1,453	12,360	43	105
Check	0	—	—	0	0	2,936	57	94

Otsego and Delaware Counties[3] were treated aerially with 1¼ pounds of technical Sevin per acre. The total area involved in this program was 85,560 acres but an attempt was made to treat only wooded land. Both Stearman and TBM-type planes were used. The treated area contained several active dairy farms.

The colonies used in these experiments were first examined on May 5 and were moved to the sprayed area on May 14 at which time traps devised by Gary (1960) for collecting dead bee bodies were placed on colony entrances.

Twenty-one colonies of honey bees of varying populations were placed in five areas within the treated area. A check apiary of five colonies was located 3¼ miles north of the sprayed area. Apiaries 1, 2, and 3 were in a valley where there was very active dairy farming. The fourth apiary was located in a heavily wooded section in a small valley where there were several dairy farms. The fifth apiary was in an area where bee forage was largely weeds and brush.

During the later part of May wild apple (*Pyrus* sp.), dandelion (*Taraxacum officinale* Weber), wild cherry (*Prunus* sp.), and yellow rocket (*Barbarea vulgaris* R. Br.) could be found in considerable quantity over the whole area. In early June alsike clover (*Trifolium hybridum* L.), devil's paintbrush (*Hieracium aurantiacum* L.), and blackberry (*Rubus* sp.) were the predominate flowers.

During the spraying operations glass plates were placed in and near apiary sites and water samples were taken from nearby streams. Analyses of these samples showed that the deposits were satisfactory for gypsy moth control.

The mortality figures given in table 1 represent those dead bees obtained by daily collections from the dead bee traps between May 19 and July 5. By the introduction of marked dead bee bodies inside the tops of colonies, it was determined that approximately 88.9% of worker bee bodies were recovered in the traps. The number of gallons of spray deposited cannot be associated closely with bee losses since the planes attempted to deposit spray on wooded areas only. The smaller Stearman-type planes were more efficient in this respect than the larger TBM planes.

The mean distance of spray application from the apiaries appears closely correlated with bee mortality. In this experiment bee losses did not occur when spray applications were made more than 1.6 miles from the apiary site.

Abnormally high bee mortalities occurred up to 3 weeks following an application of spray. Many young worker bees were recovered from the traps. Under normal conditions these young workers would not have been foraging in the field. It would appear that bees foraging at the time of spraying might have collected contaminated pollen which when consumed by hive bees could have been responsible for the continued mortalities.

All colonies were examined on June 12, July 13, and September 20, 1959. Five of the twenty-one colonies had infections of European foulbrood when examined on June 12. Their condition gradually improved and by early September all colonies were free from the disease. The relationship of this disease to the insecticide applications was not clear in these experiments and therefore further work is underway on this subject. Aside from brood losses from European foulbrood, no abnormal brood mortalities were observed during these experiments.

All colonies recovered rapidly, with the exception of four colonies where queens were lost and not replaced. Seasonal honey production (table 1) approached normal, with the exception of apiary 3. Honey production conceivably could have been affected severely if the period following the spraying had corresponded to a major honey flow. However, in this experiment the major honey flow occurred in late August and early September after the colonies had recovered.

On May 25th a commercial apiary containing 30 established colonies and 17 new (new meaning colonies started in the spring of 1959) colonies were accidentally sprayed at about 12 noon. These colonies were examined on July 16th and the honey removed. The apiary did not produce so well as other unaffected apiaries nearby and the beekeeper was compensated for the loss of about a ton of honey. There was some brood dead in many of the colonies and it would appear this brood had been chilled. No European foulbrood was found in any of the colonies. The brood pattern in most of the colonies was good.

CONCLUSIONS.—In the case of the use of Sevin, losses are readily detectable at the hive indicating that few bees are unable to return to the hive.

We believe pollen becomes contaminated with Sevin in the field and this pollen may be collected and stored without immediate adverse effect upon the pollen-collecting bees. This contaminated pollen can cause an abnormal,

[3] The assistance of the following persons is greatfully acknowledged: R. L. Ghent and R. H. Anderson, graduate students in entomology; Dr. M. H. J. Weiden; Ed Eckess and Vern O'Dell, Plant Pest Control Division, U. S. Department of Agriculture.

though not severe, loss of young bees for up to 3 weeks. Further work is in progress on this subject.

Direct applications of insecticide to the hives is not so important as contamination of the field area and plants being visited by the bees.

In this experiment no losses occurred when spraying was more than 1.6 miles away from the hives.

Colonies recovered well in this experiment and there were no serious long range effects.

REFERENCE CITED

Gary, N. E. 1960. A trap to quantitatively recover dead and abnormal honey bees from the hive. Jour. Econ. Ent. 53: 782–5.

Effects of Various Soil Types and Methods of Application upon Uptake of Three Systemic Insecticides by Cotton Plants in the Greenhouse[1]

MOHIB ZAKI[2] and H. T. REYNOLDS[3]

ABSTRACT

Plant uptake, persistence, and insecticidal efficiency of three systemic insecticides (phorate, demeton, and dimethoate) were studied on cotton plants in the greenhouse. Materials were applied as seed treatments and as granulated formulations (on attaclay and vermiculite carriers) at time of planting and to established plants. Four soil types were included: fine sandy loam (Moreno, pH 7.1); loamy fine sand (Indio, pH 9.1); clay loam (Imperial Valley, pH 8.1); and silty loam (San Marcos, pH 6).

All seed treatments retarded germination, especially in the lighter soils; soil treatments at time of planting produced the same trend but less severely. Phytotoxicity was most pronounced with seed treatments; the effects from insecticides on vermiculite granules applied at planting were more severe than from those on clay granules. Plant injury was more severe in lighter soils. The injury caused by dimethoate seed treatment was the most severe, with phorate- and demeton-caused injury following in order of decreasing intensity. Granulated formulations applied to the soil on established plants gave typical systemic burn on the leaf margins that was most severe on dimethoate-treated plants.

Bioassay with spider mites, *Tetranychus cinnabarinus* (Boisd.), revealed highest initial concentrations of toxicants in the plants grown from treated seed. The light soils allowed the fastest uptake of toxicants and the highest levels of toxicant concentration within the plants. Vermiculite granules released toxicants more rapidly than clay granules but residual activity did not persist as long. Granulated dimethoate reached lethal concentrations in the plant most rapidly but lost its effect most quickly; phorate showed the greatest persistence.

Soil pH did not prove to be a major factor in toxicant uptake, but soil texture was important, the lighter, sandy soils allowing materials to be absorbed by plant roots more rapidly. Leaching experiments demonstrated the faster movement of the systemic insecticides in sandy soils. Similar experiments demonstrated a quicker release of toxicants from vermiculite than from clay granules.

Research with various methods of applying systemic compounds for cotton insect control has been in progress for several years. Parencia *et al.* (1957a, 1957b), Stanley & Breeland (1957), Hopkins *et al.* (1958), Hanna (1958), Adkisson (1958), Dobson (1958), and Mistric & Spyhalski (1959) demonstrated the ability of seed treatments with systemic insecticides to kill certain pests on seedling plants. Reynolds *et al.* (1957) reported that applying systemics to cotton, alfalfa, and sugar beets at time of planting protected the aerial parts of these young crops from attack by various species of seedling pests.

Radiotracer studies showed that the highest concentration of systemic insecticides following seed treatments occurred in the cotyledons and that the toxicants were not translocated in substantial amounts from this point to other plant parts. Thus, continuous absorption and translocation must occur in order to protect newly formed portions of the plant (Reynolds *et al.* 1957).

In addition to seed treatment, some work was carried out with application of granulated systemic insecticides at time of planting. Reynolds *et al.* (1957) showed that the absorption and translocation following such a method of application might occur a little more slowly than with seed treatments, but the residual period of activity was somewhat longer. They also reported that in the case of intolerant seed, granules did not impair germination as seed coatings did.

Application of granulated systemic insecticides to established stands of alfalfa showed promise in controlling the spotted alfalfa aphid (*Therioaphis maculata* (*Buckton*)) (Dobson 1958). This method of application also eliminated some of the hazards of spraying or dusting.

As to the effect of soil type on the uptake of systemic insecticides by plants, several investigators reported that greater absorption occurred from sand and sandy soils than from loam and muck (David & Gardiner 1951, David 1951, Getzin & Chapman 1959, 1960).

The purpose of the present investigation was to study uptake, persistence, and relative insecticidal efficiencies of three systemic insecticides. Applications were made as seed treatment and as granulated formulations on attaclay and vermiculite carriers. The various treatments were applied to different types of soils at time of planting and to well-established plants as side dressing at a later

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[2] Faculty of Agriculture, Ain Shams University, Cairo, Egypt, U.A.R. Appointment supported by the International Cooperation Administration under the Visiting Research Scientist Program administered by the National Academy of Sciences of the United States of America.

[3] University of California Citrus Experiment Station, Riverside.

date. Laboratory experiments were conducted also to obtain preliminary information on the rate of leaching with water on certain insecticides through the various soil types.

MATERIALS AND METHODS.—*Types of Soil.*—Greenhouse experiments were undertaken using cotton plants grown in clay pots (6 inches in diameter). Four types of soil, collected from different locations in southern California, were used: fine sandy loam (Moreno, pH 7.1); loamy fine sand (Indio, pH 9.1); clay loam (Imperial Valley, pH 8.1); and silty loam (San Marcos, pH 6). All treatments were replicated three times and a check was maintained for each treatment. Plants were watered every other day with 150 ml. of tap water.

Seed Treatment.—Technical phorate, demeton, and dimethoate were each formulated in the laboratory as a 50% compound on activated charcoal for use as seed treatment. As reported by Reynolds *et al.* (1957), the seed were treated in small containers at the rate of 1 pound actual toxicant per 25 pounds of seed, allowed to dry, and then planted in the various soils at the rate of three seeds per pot.

Granulated Formulations.—For testing effects of granulated formulations used at planting time, 0.365 gram of each formulation of attaclay and vermiculite granules (5% concentration) was applied to the soil at a depth of 1 inch. This rate of application approximates 1 pound of toxicant per acre based upon a 40-inch row space. Two seeds per pot were planted directly over the granules and covered with soil.

The same amount of granules (0.365 gram) was applied as side-dress treatments to 6-week-old cotton seedlings. The granules were placed 1 inch deep and 1 inch away from the established plants in 8 equidistant holes dug with the aid of a cork borer.

Absorption and Persistence.—To evaluate the rate of absorption and the relative persistence of the above insecticides in different soils, mites (*Tetranychus cinnabarinus* (Boisd.)) were placed on the lower surface of the true leaves and kept in place by using small "clip-on" leaf cages specially designed for this purpose. Four replications of 20 mites each were allowed to feed on the treated plants for a period of 36 hours. Counts of dead mites taken at the end of this period and expressed in terms of percentage mortality indicated the rate of absorption. Counts were taken every 4 days, starting with the appearance of the true leaves, until the percentage mortality decreased to about 10% or less. In the side-dress applications, the first counts were taken 4 days after treatment, and counts were repeated every 4 days for a period of 11 weeks.

Leaching.—To obtain data on the rate of leaching of the above-named insecticides through various soils by water, 40 grams of each soil type were placed in individual tubes measuring 3×1.25 inches. The bottom of each tube was fitted with a fine-mesh muslin cloth to keep the soil in place. Preliminary tests with various amounts of formulations indicated that 20 mg. of each insecticide were suitable for the test. Accordingly, this amount was broadcast over the surface of the soil and covered with 10 additional grams of the same type of soil. Enough water was added every other day to each tube to approximate moisture content in the field. Three replicates for each treatment were maintained for periods of 0, 1, and 2 weeks. At the end of each period, each replicate was leached again with 100 ml. tap water added in 10 ml. portions. The replicates were kept at 75° to 80° F. since temperature affects the speed of release of some toxicants (Mulla & Axelrod 1960a).

Filtrates were collected in small jars and then pH values were checked. For a quantitative measurement of the insecticidal residues, 20 ml. of each clear filtrate were sufficient. A bioassay technique was used in which the 20 ml. samples were added to each of three 1-pint cups containing 80 ml. of tap water and 25 fourth-instar larvae of *Culex quinquefasciatus* Say. Mortalities of the mosquito larvae were recorded 24 hours later. The parts per million present in each cup were determined from standard dosage-mortality lines established against the test insect according to the technique described by Mulla & Axelrod (1960b).

RESULTS AND DISCUSSION.—*Germination.*—Germination counts were started 7 days after planting. Counts were made daily until the number of seedlings remained reasonably constant. At this time it was assumed that all the viable seeds had germinated.

The data in table 1 show that all the insecticidal treatments in the various soils retarded germination and emergence considerably. Following seed treatment, the small plants grown in loamy fine sandy (Indio) soil showed the most adverse effect, possibly from the combined effect of rapid toxicant uptake in the light soil and high pH (9.1). Counts taken at an intermediate date about 8 to 10 days from planting showed that of the various treatments phorate caused the least effect.

Demeton-treated seed retarded germination and emergence for about 2 or 3 days in Indio soil, but 9 days later all the treated seed had germinated and emerged. There was no difference in the rate of germination 14 days after planting between the Imperial Valley and Moreno soil treatments.

Dimethoate adversely affected germination the most and significantly reduced the number of seedling survival. The effect in different soils in increasing order of plant survival was Indio soil < Moreno soil < San Marcos soil < Imperial Valley soil.

Soil treatments at time of planting produced more or less the same results as seed treatments. Ten days after planting, phorate on attaclay and vermiculite carriers had had no effect on either germination or emergence in any of the soils with the possible exception of the Moreno soil in which 88% to 92% plant emergence was obtained.

Phytotoxicity.—A study of plant growth subsequent to emergence showed continuous high phytotoxicity in plants treated with dimethoate. Severe injury to the cotyledons and an obvious stunting of growth occurred. The injury was more pronounced with seed coatings and with vermiculite granules applied at planting time than with attaclay granules. The average height of stands, 1, 2, and 6 weeks after the development of the true leaves of the check plants, are given in table 2.

The side-dress or soil treatment to established plants produced little phytotoxicity 1 week after the treatment, although dimethoate-treated plants showed some effect.

Table 1.—Effect of seed coatings[a] and soil treatments[b] at planting time on the percentage of germination and the speed of emergence of seed planted in four soil types.

Materials and Carriers Used[c]	Per Cent Germination of Seed on Days Shown After Planting															
	Indio Soil (loamy fine sand, pH 9.1)				Imperial V. Soil (clay loam, pH 8.1)				Moreno Soil (fine sandy loam, pH 7.1)				San Marcos Soil (silty loam, pH 6)			
	8	10	12	14	8	10	12	14	8	10	12	14	8	10	12	14
Seed Coatings																
Phorate	50	91	100	100	100	100	100	100	62	82	92	92	92	100	100	100
Demeton	0	42	100	100	92	92	92	92	25	82	92	92	83	100	100	100
Dimethoate	0	0	11	33	40	56	70	100	0	9	25	30	0	66	83	83
Check	83	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Soil Treatments																
Phorate on At.	100	100	100	100	72	100	100	100	62	82	82	92	87	100	100	100
Phorate on V.	100	100	100	100	100	100	100	100	60	82	88	88	75	100	100	100
Demeton on At.	0	50	100	100	72	100	100	100	0	37	82	88	75	100	100	100
Demeton on V.	0	75	87	87	72	100	100	100	9	36	72	88	87	100	100	100
Dimethoate on At.	0	0	62	62	0	38	72	100	0	0	25	62	0	0	38	100
Dimethoate on V.	0	12	62	62	0	25	62	100	0	0	12	45	0	0	87	90
At. alone (check)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
V. alone (check)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

[a] Seed treated at the rate of 1 pound of toxicant per 25 pounds of seed. Materials formulated as 50% toxicant on activated charcoal.
[b] Soil treated at equivalent rate of 1 pound of toxicant per acre.
[c] At. indicates the materials were formulated on attaclay and v. on vermiculite in all tables.

Two weeks later phytotoxicity was visible in both phorate and dimethoate treatments in Moreno and Indio soils. Moreover, the dimethoate, particularly the vermiculite formulation, by this time showed severe injury to the lower leaves and was easily distinguished from the other treatments. Demeton showed the least phytotoxic effect. On the other hand, cotton plants grown in the Imperial Valley and San Marcos soils recovered from earlier phytotoxicity effects 4 weeks after the treatments and compared favorably with the untreated check plants. Growth observations of the treated and untreated plants indicated that phorate may have stimulated somewhat the plant growth, particularly in Moreno soil under the greenhouse conditions of these tests.

Effect on Mites.—As shown in table 3, the percentage mortality of spider mites revealed higher concentrations of the insecticides in the seedlings produced from seed treatments than in those produced from seed in treated soils. Phorate seed treatments proved to be highly effective (55% to 100% mortalities) until the tested seedlings were about 7 weeks old and showed a fair degree of protection (40% or higher mortalities) for 1 more week. The order of uptake of phorate by the plants following seed treatment from the various soils was as follows: Indio > Moreno > Imperial Valley > San Marcos.

Demeton seed treatments resulted in a high degree of protection until the tested seedlings were about 5 weeks old. The absorption was greater in Indio and Moreno soils, but was less than that obtained with phorate. As the result of plant injury and stunting, dimethoate uptake

Table 2.—Effect of seed coatings[a] and soil treatments[b] at planting time on the average height of plants in various soils, 1, 2, and 6 weeks after the development of the first true leaves.

Materials and Carriers Used	Average Height of Plants (inches) After Weeks Indicated											
	Indio Soil (loamy fine sand, pH 9.1)			Imperial V. Soil (clay loam, pH 8.1)			Moreno Soil (fine sandy loam, pH 7.1)			San Marco Soil (silty loam, pH 6)		
	1	2	6	1	2	6	1	2	6	1	2	6
Seed Coatings												
Phorate	2	3	6	3	4	10	2.5	4	8	2.5	3	10
Demeton	1	2	7	2	2.5	8	1	2	7	1.5	3.5	8
Dimethoate	—	0.5	2	0.5	1	4	—	1.5	3	1	2	5
Check	4	4.5	9	4.5	5	13	2.5	5	11	4	4.5	13
Soil Treatment												
Phorate on At.	3	3.5	6	3.5	4.5	10	3	4	6	3	4.5	11
Phorate on V.	2.5	4.5	7.5	3	5	13	2	4	8	2	4	10
Demeton on At.	1.5	2.5	4	2	3.5	9	1.5	3	4	2.5	3.5	8
Demeton on V.	1.5	2.5	8	2.5	3	10	1.5	3	9	2	3	9
Dimethoate on At.	0.5	1.5	3	2	3	7	1	1.5	2	1	1	3
Dimethoate on V.	0.5	2	4.5	2	3	9	0.5	2	3.5	1.5	2	6
Check	4	4.5	9	4.5	5	13	2.5	5	11	4	4.5	13

[a] Same as for table 1. [b] Same as for table 1.

Table 3.—Effect of seed coating[a] and soil treatments[b] on planting time on the uptake of three insecticides from various soils, indicated as percentage mortality of mites.

Materials and Carriers Used	Per Cent Mortality of Mites on Days Shown After Seed Germination																			
	Indio Soil (loamy fine sand, pH 9.1)					Imperial V. Soil (clay loam, pH 8.1)					Moreno Soil (fine sandy loam, pH 7.1)					San Marcos Soil (silty loam, pH 6)				
	30	38	46	54	62	30	38	46	54	62	30	38	46	54	62	30	38	46	54	62
Seed Coatings																				
Phorate	100	100	85	40	10	90	90	80	30	5	100	100	70	30	5	100	85	55	25	10
Demeton	100	90	65	20	5	90	60	45	25	10	95	85	40	25	5	100	75	35	20	0
Dimethoate	—	—	—	10	0	—	—	30	25	5	—	—	20	5	0	—	—	25	10	5
Check	0	0	0	0	0	0	0	0	0	0	0	0	10	0	10	0	0	10	5	0
Soil Treatments																				
Phorate on At.	85	55	45	20	15	90	70	50	30	10	85	70	55	15	10	100	90	60	25	5
Phorate on V.	90	65	60	20	5	100	75	75	20	10	90	90	50	25	5	100	85	70	30	0
Demeton on At.	—	90	60	20	15	95	85	45	15	10	100	85	50	30	0	100	90	35	30	5
Demeton on V.	—	90	65	35	5	90	70	45	25	0	80	85	45	25	5	90	90	65	25	0
Dimethoate on At.	—	—	25	15	10	—	—	15	5	5	—	—	25	20	10	—	—	25	15	10
Dimethoate on V.	—	—	30	25	5	—	—	30	10	0	—	—	40	15	0	—	—	25	10	0
At. alone check	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	10	0	0
V. alone check	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0

[a] Same as for table 1. [b] Same as for table 1.

from the various soils was not checked until the seedlings were 6 to 8 weeks old and apparently the toxicant was deteriorating by this date.

Phorate and demeton soil treatments at planting time showed more or less the same degree of absorption from the different soils. In most cases the vermiculite formulations gave a higher degree of protection initially than the attaclay formulations under the same experimental conditions. The uptake of dimethoate was not examined until the seedlings were 5 to 6 weeks old. The order of uptake of dimethoate from the various soils was as follows: Moreno > Indio > Imperial Valley > San Marcos.

Side-dress applications to established cotton plants grown in the various soils produced rather high and somewhat comparable levels of toxicity to mites (60% to 100% mortality) 12 days after treatment (table 4). In general initial toxicities with vermiculite formulations were higher (about 8%) than attaclay formulations. Again, dimethoate showed the highest initial toxicity with a maximum of 100% mortality in Moreno and Indio soils.

In the same soils demeton and phorate also gave 100% mite mortalities although they both took a little longer to attain this degree of kill. In the two heavier soils (Imperial Valley and San Marcos) the degree of mortalities obtained were with some exceptions neither as high nor quite as persistent.

Plants treated with phorate and demeton retained some degree of toxicity for about 64 or more days, the phorate-treated plants yielding slightly higher kill than the demeton. Attaclay formulations of dimethoate persisted slightly longer than vermiculite formulations, but persistence with this material was considerably shorter than that obtained with phorate and demeton.

During the course of the investigation, the pH value of the treated soil did not prove to be a major factor in the uptake of the three insecticides by cotton plants. Plants grown in Moreno soil (pH 7.1) showed the highest measure of protection followed by those in Indio soil (pH 9.1). Plants in the Imperial Valley (pH 8.1) and San Marcos (pH 6) soils came in the third and fourth positions insofar as protection is concerned. Under the same conditions, vermiculite formulations were more effective initially with all the materials in controlling spider mites than the attaclay formulations. The reason for this difference appears to be due to quicker release of the toxicants from the vermiculite carrier. As might be expected, the effectiveness of vermiculite formulations declined more rapidly.

It was also observed that the soil texture is probably an important factor in the uptake of the insecticides from soils. The order of efficiency of the insecticides in various soils was Moreno soil (fine sandy loam) > Indio soil (loamy fine sand) > Imperial Valley soil (clay loam) > San Marcos soil (silty loam).

Rate of Leaching in Soils.—Inspection of results in table 5 reveals several points of interest. The materials formulated on vermiculite leached more readily than those formulated on attaclay. This fact, no doubt, largely accounts for results presented earlier which showed that mite mortalities occurred a little more rapidly with vermiculite formulations than with attaclay. Further, the data in table 5 show that the rate of leaching (and thus movement in the soil into plant root zone) is directly proportional to water solubility of chemicals involved. The relative rates of leaching of the materials tested were as follows: dimethoate > demeton > phorate. Results obtained also confirm the observation that the materials move more rapidly in light soil. The relative rates of movement in the soil were as follows: loamy fine sand (Indio soil) > fine sandy loam (Moreno soil) > silty loam (San Marcos soil) > clay loam (Imperial Valley soil).

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Table 4.—Effect of side-dress treatments on the uptake of three systemic insecticides from various soils, indicated as percentage mortality of mites.

Materials and Carriers Used[a]	Per Cent Mortality of Mites on Days Shown After Side-Dressing													
	12	16	20	24	28	32	36	40	44	48	52	56	60	64
Indio Soil (Loamy fine sand, pH 9.1)														
Phorate on At.	60	100	100	100	93	75	72	60	53	60	60	60	40	40
Phorate on V.	70	100	100	100	100	83	73	66	53	48	63	60	48	33
Demeton on At.	73	100	100	100	80	60	66	53	50	40	48	33	30	30
Demeton on V.	80	100	100	100	75	63	60	53	48	33	43	30	26	20
Dimethoate on At.	100	100	100	100	60	36	33	26	16	23	20	16	20	—
Dimethoate on V.	100	100	100	83	53	26	23	20	20	16	6	—	—	—
Imperial Valley Soil (Clay loam, pH 8.1)														
Phorate on At.	85	86	90	90	93	83	66	63	60	68	50	50	33	30
Phorate on V.	83	90	93	100	93	90	73	66	66	60	48	33	30	26
Demeton on At.	70	86	90	100	83	76	66	66	53	50	56	50	40	26
Demeton on V.	75	93	90	100	93	83	73	66	60	50	48	53	26	30
Dimethoate on At.	80	90	83	90	90	70	40	43	40	36	20	5	—	—
Dimethoate on V.	80	93	90	76	90	63	48	30	23	23	16	—	—	—
Moreno Soil (Fine sandy loam, pH 7.1)														
Phorate on At.	80	100	100	100	100	100	86	80	70	60	56	56	48	53
Phorate on V.	83	100	100	100	100	93	93	83	73	66	60	63	53	50
Demeton on At.	86	83	93	100	100	86	83	70	66	60	53	48	48	40
Demeton on V.	93	86	100	100	100	100	86	86	70	66	66	63	53	36
Dimethoate on At.	86	100	100	100	63	60	60	53	36	40	26	15	10	—
Dimethoate on V.	100	100	83	80	60	50	44	40	33	23	15	15	—	—
San Marcos Soil (Silty loam, pH 6)														
Phorate on At.	70	73	93	90	70	70	63	60	60	53	50	33	26	16
Phorate on V.	80	90	93	93	90	73	70	63	55	50	50	33	30	16
Demeton on At.	60	70	73	83	83	70	70	50	50	40	36	26	—	—
Demeton on V.	66	76	76	86	83	72	66	48	53	36	36	30	16	—
Dimethoate on At.	80	80	83	100	80	70	33	26	30	10	10	—	—	—
Dimethoate on V.	83	90	80	93	76	55	33	30	30	10	5	—	—	—
Check	—	5	—	10	—	—	—	—	—	—	—	—	—	—

[a] Soil treated at an approximate rate of 1 pound of toxicant per acre.

Table 5.—Residues leached from various soils by water at weekly intervals after treatments.

Materials and Carriers Used	Residues Leached (p.p.m.)											
	Moreno Soil			Indio Soil			Imperial Valley Soil			San Marcos Soil		
	0	1	2	0	1	2	0	1	2	0	1	2
Phorate on At.	0.024	0.020	0.015	0.022	0.018	0.016	0.016	0.015	0.014	0.010	0.010	—
Phorate on V.	.032[a]	.024	.017	.030	.024	.017	.018	.017	.016	.012	.010	—
Demeton on At.	.600[a]	.460	.260	.410	.360	.250	.340	—	—	.250	—	—
Demeton on V.	.600[a]	.440	.360	.420	.370	.270	.390	.240	—	.270	—	—
Dimethoate on At.	.720	.690	—	.840	.500	.340	.590	—	—	.620	.440	—
Dimethoate on V.	.900[a]	.720	—	.900[a]	.450	—	.690	.440	—	.740	.460	—

[a] Beyond range of mortality curve (100% kill of mosquito larvae).

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Toxicity to House Fly Larvae of Droppings from Chicks Administered Insecticides in Feed, Water, and as Single Oral Dosages[1]

MARTIN SHERMAN and ERNEST ROSS[2]

ABSTRACT

The toxicity to house fly (*Musca domestica* L.) larvae of droppings from chicks administered the following 23 insecticides was determined: American Cyanamid 12008 (*O,O*-diethyl *S*-isopropylthiomethyl phosphorodithioate), American Cyanamid 18706 (*O,O*-dimethyl *S*-(*N*-ethylcarbamoylmethyl) phosphorodithioate), Bayer 22408 (*O,O*-diethyl *O*-naphthaloximidophosphorothionate), Bayer 25141 (*O,O*-diethyl *O*-*p*-(methylsulfinyl) phenyl phosphorothioate), Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), DDVP, Delnav® (2,3-*p*-dioxanedithiol *S, S*-bis (*O,O*-dimethyl phosphorodithioate)), dicapthon, dimethoate, Dowco 105 (*O*-methyl *O*-(4-*tert*-butyl-2-chlorophenyl) ethylphosphoramidothioate), Dowco 109 (*O*-(4-*tert*-butyl-2-chlorophenyl)*O*-methyl methylphosphoramidothioate), heptachlor, Isolan® (dimethyl 5-(1-isopropyl-3-methylpyrazolyl) carbamate), Kepone® (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd] pentalen-2-one), Perthane® (1,1-dichloro-2, 2-bis (*p*-ethylphenyl)ethane), Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate), phosphamidon, Pyrolan® (dimethyl 5-(3-methyl-1-phenylpyrazolyl) carbamate), Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate), Sevin® (1-naphthyl *N*-methylcarbamate), Strobane® (a mixture of chlorinated terpenes with about 66% Cl (chlorinated α-pinene isomers)), and *Bacillus thuringiensis* var. *thuringiensis* Berliner.

Feces collected daily from chicks given single oral dosages of insecticides caused greater than 90% mortality in house fly larvae for varying periods of time as follows: Bayer 22408, 6 days; dicapthon, 5 days; dimethoate, 4 days; Dowco 105, 2 days; *Bacillus thuringiensis*, 1 day; Co-Ral, 1 day; Dowco 109, 1 day; and Perthane, 1 day. Single oral dosages of American Cyanamid 12008, American Cyanamid 18706, Bayer 25141, Bayer 29493, DDVP, Delnav, heptachlor, Isolan, Kepone, Phosdrin, phosphamidon, Pyrolan, Ruelene, Sevin, and Strobane caused little or no mortality in house fly larvae placed, within 24 hours, in droppings from treated chicks.

The insecticide levels in the feed that caused greater than 80% mortality in larvae placed in the manure were: American Cyanamid 12008, 110 p.p.m.; American Cyanamid 18706, 220 p.p.m.; Bayer 22408, 22 p.p.m.; Bayer 25141, 80 p.p.m.; Bayer 29493, 220 p.p.m.; dicapthon, 440 p.p.m.; dimethoate, 44 p.p.m.; and phosphamidon, 440 p.p.m. Somewhat less toxic to the larvae were: DDVP, Delnav, Kepone, and Pyrolan. Of little or no toxicity to the larvae at the highest concentration level tested were: Dowco 105, Dowco 109, Isolan, Perthane, Ruelene, Sevin, and *Bacillus thuringiensis*.

Dimethoate and phosphamidon administered to chicks in the drinking water at 22 and 220 p.p.m., respectively, resulted in greater than 90% mortality in larvae placed in the droppings. Chicks given solutions of 220 p.p.m. Isolan produced droppings which were nontoxic to house fly larvae.

The feeding of insecticides to poultry has been shown to be successful in preventing development of house fly (*Musca domestica* L.) larvae in manure. These insecticides can be administered in feed, water, or as single oral doses. Sherman & Ross (1959) found that materials excreted as late as 6 days after chicks were fed a single oral dose of insecticide were still sufficiently toxic to kill more than 90% of the house fly larvae placed in this manure. A similar manure toxicity was demonstrated when insecticides were added to the feed at levels as low as 89 p.p.m. and fed to poultry over a period of time (Sherman & Ross 1960a). When insecticides were dissolved in the water supply of poultry, a mode of administration that has several advantages over the other two methods, Sherman & Ross (1960a) found that Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) at a concentration of 30 p.p.m. in distilled or acidified water caused high levels of larval mortality in the droppings. These results have demonstrated the feasibility of controlling fly breeding in manure by oral ingestion of low levels of insecticides. Therefore, an intensive program to screen candidate materials potentially effective as larvicides was undertaken. This report describes the larvicidal effectiveness of 23 insecticides after administration as single oral dosages, as feed additives, or as solutions. The effect of these treatments on the chicks, with special emphasis on acute and sub-acute toxicity, is discussed in detail by Sherman & Ross (1961).

METHODS AND RESULTS.—The Hawaii strain of the New Hampshire breed of chicks was used in this study. All chicks received, to 7 or 8 days of age, a starter ration consisting of the following composition: soybean oilmeal, 36%; ground corn, 60%; defluorinated phosphate, 3%; iodized NaCl, 0.5%; and vitamins and trace minerals, 0.5%. After this time, the chicks were distributed at random into experimental groups and started on their respective treatments. Feed, water, and light were furnished continuously. The chicks were housed in thermostatically controlled starter batteries with wire floors to control coprophagy.

The composition of each of the 22 chemical insecticides studied in this investigation is listed in table 1. In addition, the effect of *Bacillus thuringiensis* var. *thuringiensis* Berliner was also studied. The *B. thuringiensis* formulation was a wettable powder containing 70 billion spores per gram.

Droppings were collected and tested for larval toxicity by the methods previously described by Sherman & Ross (1959, 1960a, 1960b). All larval mortality was corrected for natural mortality by Abbott's formula (Abbott 1925). Mortality of house fly larvae in the manure from the untreated control animals was low, ranging from 0% to 20%.

Single Oral Dosages.—Standard stock solutions of the technical grade materials and suspensions of the wettable powder formulations were prepared in acetone and dis-

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[2] Entomologist and Associate Poultry Scientist, respectively.

Table 1.—The composition of insecticides tested.

NAME	CHEMICAL DEFINITION	FORMULATION USED
Am. Cyanimid 12008	*O,O*-diethyl *S*-isopropylthiomethyl phosphorodithioate	Technical
Am. Cyanamid 18706	*O,O*-dimethyl *S*-(*N*-ethylcarbamoylmethyl) phosphorodithioate	Technical, m.p. 65.0–65.6° C.
Bayer 22408	*O,O*-diethyl *O*-napthaloximidophosphorothionate	Technical, m.p. 151–156° C.
Bayer 25141	*O,O*-diethyl *O*-*p*-(methylsulfinyl)phenyl phosphorothioate	Technical
Bayer 29493	*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate	Technical
Co-Ral®	*O*-(3-chloro-4-methylumbelliferone)*O,O*-diethyl phosphorothioate	Technical, 97.0%, m.p. 93.5–94.5° C.
DDVP[a]		Technical, 100%
Delnav®	2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-dimethyl phosphorodithioate)	Technical
Dicapthon[a]		Technical, 96%, m.p. 48.5–49.0° C.
Dimethoate[a]		Technical, m.p. 40.5–42.5° C.
Dowco 105	*O*-methyl *O*-(4-*tert*-butyl-2-chlorophenyl) ethylphosphoramidothioate	25% wettable powder
Dowco 109	*O*-(4-*tert*-butyl-2-chlorophenyl) *O*-methyl methylphosphoramidothioate	25% wettable powder
Heptachlor[a]		Technical, 72%, m.p. 57–60° C.
Isolan®	Dimethyl 5-(1-isopropyl-3-methylpyrazolyl) carbamate	Technical
Kepone®	Decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd] pentalen-2-one	Technical, m.p. 294–299° C.
Perthane®	1,1-dichloro-2,2-bis (*p*-ethylphenyl)ethane	Technical
Phosdrin®	1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate	Technical, 60%
Phosphamidon[a]		Technical, 88.8%
Pyrolan®	Dimethyl 5-(3-methyl-1-phenylpyrazolyl) carbamate	Technical, m.p. 47.5–49.0° C.
Ruelene®	*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate	25% wettable powder
Sevin®	1-naphthyl *N*-methylcarbamate	50% wettable powder
Strobane®	A mixture of chlorinated terpenes with about 66% Cl (chlorinated α-pinene isomers	Technical, 100%

[a] Common name approved by the Committee on Insecticide Terminology of the Entomological Society of America.

tilled water, respectively. The required dosage of insecticide was administered either by gelatin capsule in the case of the technical grade insecticides or by means of a pipette in the case of the wettable powder suspensions (Sherman & Ross 1959). Dosage was calculated in terms of milligrams of technical toxicant per kilogram of chick body weight. Droppings were collected daily for 7 days and tested for larval toxicity.

Several of the insecticides, when fed as single oral dosages to the chicks, caused the droppings to be highly toxic to the house fly larvae. Figure 1 gives a summary of the larval mortality caused by the six insecticides that were most effective. Bayer 22408 at a level of 1000 mg./kg. caused 96% mortality in first-instar larvae 6 days after treatment. Dimethoate at 30 mg./kg., dicapthon at 150 mg./kg. and Dowco 105 at 1500 mg./kg. caused 100% larval mortality for 2 days or longer. Co-Ral® at 12.5 mg./kg. and Perthane® at 2000 mg./kg. caused high larval mortality 24 hours after chick treatment, but this mortality decreased appreciably by the second day.

Bayer 22408, dimethoate, dicapthon, Dowco 105, Co-Ral, and Perthane at the above-mentioned dosage levels caused 0%, 30%, 20%, 60%, 40%, and 0% chick mortality, respectively.

Because of the low larval mortality occurring after feeding most of the other insecticides to the chicks, manure collections were discontinued after 2 or 3 days of chick treatment. Table 2 summarizes data on the toxicity of these insecticides to house fly larvae. Dowco 109 and *Bacillus thuringiensis* at 450 and 1000 mg./kg., respectively, caused high larval mortality 24 hours after chick treatment, but by the second day were ineffective. American Cyanamid 12008, American Cyanamid 18706, Bayer 25141, Bayer 29493, DDVP, Delnav®, heptachlor, Isolan®, Kepone®, Phosdrin®, phosphamidon, Pyrolan®, Ruelene®, Sevin®, and Strobane® (which caused low mortality in the chicks) caused little or no mortality in house fly larvae placed in droppings from treated chicks.

Feed Mixtures.—All of the insecticides mentioned above, with the exception of Co-Ral, heptachlor, Phosdrin, and Strobane, were tested by incorporation into the basal ration. This was accomplished by placing 5 pounds of the standard starter ration into a large mixing bowl, slowly adding 100 ml. of acetone solution or suspension containing the required amount of technical insecticide, and mixing by hand for 5 minutes. The mixed material was placed as a thin layer on clean paper to allow the acetone to evaporate. The *Bacillus* was suspended in 100 ml. of distilled water, added to the feed in the same manner as described above, and was allowed to air-dry for several hours before being bagged. The resultant concentrations of toxicant-feed mixtures were expressed in p.p.m. based on the amount of technical toxicant in the feed. The insecticide-treated feed was assigned to the experimental groups at random and completely replaced the basal feed. These feeds and untreated water were supplied *ad libitum* for the 7- to 14-day duration of each experiment. In sampling the droppings, no collection was made earlier than 2 days after the chicks were started on a treated diet and at least three collections, on different days, were made at each concentration level.

Table 3 summarizes data on the toxicity of the droppings, from chicks given insecticide-feed mixtures, to first-instar house fly larvae. A number of materials were not very toxic and, at the highest concentrations tested, caused less than 30% mortality in the house fly larvae placed in the manure. These materials were: Dowco 105, Dowco 109, Isolan, Perthane, Ruelene, Sevin, and *Bacillus thuringiensis*. The feeding of other insecticides produced droppings which were more toxic, but caused less than 80% mortality in the larvae at the highest concentration level in the feed. This group included: DDVP, Delnav, Kepone, and Pyrolan. The insecticides which caused the highest larval mortality in manure were:

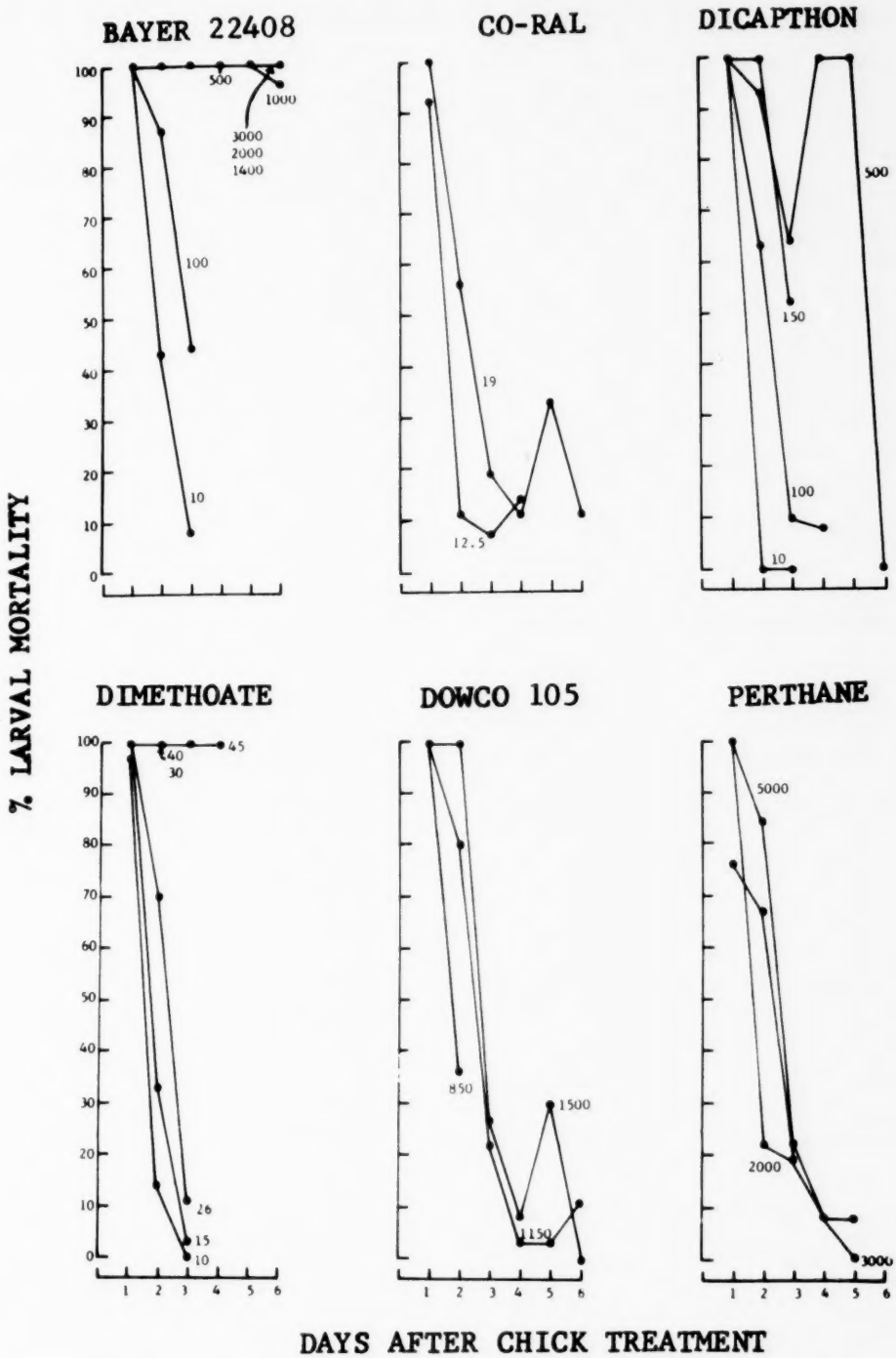

FIG. 1.—Toxicity of poultry manure, from chicks treated with a single dose of insecticide, to first-instar house fly larvae. Manure collected daily after chick treatment. Figures within graphs refer to dosage (mg./kg.) of insecticide received by chick.

Table 2.—Toxicity to house fly larvae of manure collected daily after chicks were treated with a single dose of insecticide.

Insecticide	Dosage (mg./kg.)[a]	Total No. Larvae Tested	Corrected Mean Per Cent Mortality After		
			1 Day	2 Days	3 Days
Am. Cyanamid 12008	10	30	—[b]	65	0
	1	30	7	—[b]	0
Am. Cyanamid 18706	10	60	64	7	0
	1	60	28	28	0
Bayer 25141	1	30	—[b]	0	2
Bayer 29493	10	30	—[b]	0	16
DDVP	15	60	10	10	7
	12	60	0	10	0
	10	60	7	0	22
	8	60	0	4	4
	1	60	0	0	22
Delnav	100	30	—[b]	70	0
	10	60	27	0	0
Dowco 109	450	50	90	0	17
	300	50	40	10	0
Heptachlor	65	70	32	13	20
	50	140	9	16	13
	25	120	25	18	13
Isolan	1.0	30	35	—[b]	16
	.5	30	13	—[b]	0
	.1	30	30	—[b]	0
Kepone	100	60	68	0	0
	10	60	3	0	0
	1	60	3	0	0
Phosdrin	9	30	8	16	—[b]
	8	50	43	0	—[b]
	7	40	66	0	—[b]
	6	50	8	8	—[b]
	5	120	3	6	—[b]
	3	60	0	0	—[b]
	2	60	0	3	—[b]
Phosphamidon	10	60	0	28	—[b]
	1	60	0	10	—[b]
Pyrolan	9	110	55	34	—[b]
	5	60	28	0	—[b]
	3	60	20	36	—[b]
	2	60	84	31	—[b]
	1	60	0	14	—[b]
Ruelene	150	60	25	3	0
	100	60	11	8	10
	88	60	13	0	0
Sevin	300	30	—[b]	0	0
	210	30	—[b]	0	0
	150	60	25	0	0
Strobane	190	10	—[b]	4	7
	150	10	—[b]	16	—[b]
	125	50	0	17	—[b]
	100	30	—[b]	0	—[b]
	Bacillus thuringiensis				
	1,000	60	100	14	—[b]
	100	60	0	0	—[b]
	10	60	0	0	—[b]
	1	60	0	0	—[b]

[a] Dosage given in terms of milligrams of toxicant per kilogram chick weight.
[b] No toxicity data obtained.

American Cyanamid 12008, American Cyanamid 18706, Bayer 25141, Bayer 29493, dicapthon, dimethoate, and phosphamidon. Each of these compounds caused at least 80% mortality in the house fly larvae. Relatively low levels of Bayer 22408 (22 p.p.m.), Bayer 29493 (22 p.p.m.), and dimethoate (44 p.p.m.) were highly toxic to house fly larvae via this route of administration.

Both Kepone and phosphamidon caused mortality in the chicks at levels of 440 and 220 p.p.m. None of the other insecticides tested by this route of administration caused death in the chicks during the course of the experiment.

Dunn (1960) and Harvey & Brethour (1960) have reported that *Bacillus thuringiensis*, as a feed additive, was effective in preventing house fly development in feces, with most of the flies dying in the pupal stage. The droppings from 5-pound hens whose daily intake of *B. thuringiensis* via the feed was 0.0078 g. of spores caused a 92% reduction in adult fly emergence (Harvey & Brethour 1960). This was calculated to be equivalent to a daily dosage of 986 million spores per kg. hen weight.

In our studies with feed mixtures containing *B. thuringiensis*, the house fly larvae surviving the 3-day observation period were not held for adult emergence. However, adult emergence was observed in the experiment using single oral dosages of *Bacillus. B. thuringiensis* given as a single oral dose to the chicks at the rate of 1 g. (70 billion spores) per kg. of body weight, caused 100% mortality in larvae placed in the droppings. However, at the 7 billion spores per kg. dosage level 29 of the 30 larvae placed in the manure were alive after the usual 3-day observation period. These larvae were left in the manure and held for adult emergence. Twenty adult flies emerged from this manure as compared with 23 adult flies that emerged from 29 larvae similarly held in manure from untreated chicks.

Water Solutions.—Stock solutions of technical dimethoate, Isolan, and phosphamidon were prepared and diluted with distilled water to the required concentration. The water solutions were stored in 5-gallon glass bottles atop the chick batteries and conducted to the watering troughs by rubber tubing. The solution level in the troughs were maintained by siphoning, when necessary. No other water was available to the chicks in these experiments. These solutions and untreated feed were supplied *ad libitum* for the 7-day duration of each experiment. The droppings were sampled as described above.

Table 4 summarizes the effect on the toxicity to house fly larvae of droppings from chicks given these solutions of dimethoate, Isolan, and phosphamidon in the drinking water. A solution containing 22 p.p.m. of dimethoate caused 91% mortality in larvae placed in the droppings. This same level of dimethoate in the feed produced droppings which caused only 27% larval mortality (table 3). Since chicks, at this age, will consume approximately twice as much water as feed, the intake of dimethoate from a concentration of 22 p.p.m. in solution would be equivalent to that from 44 p.p.m. in feed. This is shown by comparing the actual intake of feed and water containing dimethoate, by the chick. The chicks consumed 59 g. of solution containing 22 p.p.m. dimethoate per day and 24.5 g. of feed containing 44 p.p.m. dimethoate per day. This is further shown by comparing the 97% mortality caused by a concentration of 44 p.p.m. in the feed (table 3) with the 91% mortality caused by 22 p.p.m. in solution. A similar relationship was shown concerning the toxicity to house fly larvae of Dipterex administered in feed and water (Sherman & Ross 1960b). No chick mortality was caused by 22 p.p.m. dimethoate in water.

Isolan was ineffective at the highest concentration tested. However, this concentration was highly lethal to the chicks, causing 100% mortality within a week.

Phosphamidon at a concentration of 220 p.p.m. in

Table 3.—Toxicity to house fly larvae of manure from chicks fed insecticide-treated rations.

Insecticide	Concentration[a] in Feed Mixture (p.p.m.)	No. Larvae Tested	Corrected Mean Per Cent Mortality
Am. Cyanamid 12008	220	90	100
	110	90	100
	22	90	22
	2.2	90	17
Am. Cyanamid 18706	440	120	100
	220	120	98
	22	120	13
	2.2	120	1
Bayer 22408	440	120	100
	220	120	100
	44	90	99
	22	210	91
	11	90	22
	2.2	120	9
Bayer 25141	110	80	80
	22	90	11
	2.2	90	0
Bayer 29493	440	40	100
	220	90	100
	22	90	74
	2.2	90	0
DDVP	220	150	58
	22	150	4
	2.2	150	4
Delnav	440	90	66
	220	90	16
	110	90	2
	22	90	2
Dicapthon	440	150	90
	220	150	76
	22	150	12
Dimethoate	440	150	100
	220	240	100
	176	90	100
	88	90	100
	44	90	97
	22	150	27
Dowco 105	440	90	2
	220	90	0
	22	90	3
	2.2	90	39
Dowco 109	440	90	3
	220	90	17
	22	90	24
	2.2	90	2
Isolan	220	120	11
	22	120	11
	4.4	120	11
	2.2	120	55
Kepone	440	90	76
	220	90	15
	110	90	7
	22	90	6
Perthane	440	90	9
	220	90	1
	22	90	3
	2.2	90	2
Phosphamidon	440	10	89
	220	100	18
	22	120	18
	2.2	120	0
Pyrolan	440	90	79
	220	90	40
	22	90	13
	2.2	90	11
Ruelene	440	90	0
	220	90	0
	22	90	0
	2.2	90	6
Sevin	440	120	0
	220	120	6
	22	120	3
	2.2	120	29
Bacillus thuringiensis	220	150	10
	22	150	12
	2.2	150	8

[a] Concentrations in terms of technical insecticide.

solution caused 100% larval mortality in the droppings as compared with 18% larval mortality in droppings from chicks fed this same concentration in the feed (table 3). On the other hand, 440 p.p.m. in the feed caused 89% larval mortality. Phosphamidon at 220 p.p.m. in solution caused 100% chick mortality within a week.

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Table 4.—Effect of distilled water solutions of insecticides on the toxicity of chick droppings to house fly larvae.

Insecticide	Concentration[a] in Solution (p.p.m.)	No. Larvae Tested	Corrected Mean Per Cent Mortality
Dimethoate	440	100	100
	220	120	100
	22	210	91
	10	90	60
	5	90	9
	1	90	1
	0.5	90	0
Isolan	220	30	5
	22	120	38
	2.2	120	10
Phosphamidon	220	20	100
	22	120	16
	2.2	120	21

[a] Concentration in terms of technical insecticide.

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The Effectiveness of Chemically Treated Screens in Killing Annoying Punkies, *Culicoides obsoletus*[1]

HUGO JAMNBACK, *New York State Museum and Science Service, Albany*

ABSTRACT

A method of testing and evaluating chemicals painted on window screens for control of punkies (*Culicoides obsoletus* (Meigen)) in dwellings consisted of using test chambers made from small cartons with one end covered by transparent plastic and the other end opaque. A screen was placed in the chamber between the light and dark ends. When the adults were introduced into the chamber at the dark end they quickly migrated to the light end passing through the treated screen. A 7.7% malathion-alcohol solution painted on screens killed *Culicoides* adults that had passed through them in less than an hour while a 10% DDT-oil solution required several hours before an appreciable mortality occurred. The malathion-painted screens were still highly effective in killing exposed punkies 21 days after treatment.

Females of *Culicoides obsoletus* (Meigen) (Diptera: Ceratopogonidae) in common with some other species in the genus are attracted to light at night and often enter homes where their habit of attacking humans makes them unwelcome visitors. Because of their small size (length 1–1.5 mm.) they can readily pass through the screens commonly used in dwellings to exclude house flies and mosquitoes.

In the absence of more basic control measures, treating the screens with insecticides provides some measure of relief. Foote & Pratt (1954) summarized and added to earlier reports on formulations used for this purpose. (Also see Blanton *et al.* 1955). Mixtures of pyrethrum and lubricating oil, sometimes with kerosene added, and later DDT solutions were used to paint window screens with some success in controlling this pest.

METHODS.—Since it is difficult to evaluate the relative effectiveness of different formulations painted on screens in units as large and varied as dwellings, small test chambers were used as a substitute in our studies. Two views of the components of these chambers are shown in figure 1. The larger carton has transparent plastic at one end. When the two are joined one end is dark and is separated by a screen from the light end. The punkies to be tested were collected in an aspirator and then transferred to the dark end of the test chamber through a small temporary opening. They passed quickly from the dark end of the cylinder, through the treated screen, to the light end where they remained. Unless otherwise specified, 20 punkies were put into each chamber for testing. Aluminum 14-×18-mesh screen was used in all of the tests because it is widely available and is often used for window screening. The formulation to be tested was painted on both sides of the screen with a brush and allowed to dry for at least a day before testing.[2]

RESULTS.—The first formulation tested was a 10% DDT solution (wt./wt.) (solvents, heavy aromatic naphtha and kerosene). The screen was painted and dried for a day and then placed in a container and tested 1, 12, 13 and 16 days after being painted. Observations and counts were made after 45 minutes, 2 hours, and 12 hours. In all of these tests there was no mortality after 45 minutes, not more than 1 dead after 2 hours and 19 or 20 dead after 12 hours. In the untreated cartons mortality ranged from zero to three out of 20 punkies after 12 hours.

This DDT-treated screen was tested again after 16 days with similar results, as shown in table 1, which also shows the effectiveness of two other formulations tested at the same time.

The long period between exposure to DDT and death of *C. obsoletus* females suggests that, after passing through the screen, they might be able to take a blood meal before dying. To test this possibility five female *C. obsoletus* were collected and allowed to pass through a DDT-treated screen. They were brought into the laboratory within 25 minutes and placed in an Erlenmeyer flask which was inverted and placed over my arm. One punkie began feeding immediately and engorged normally. The flask was then removed and an hour later again placed over my arm, and a second punkie engorged normally. The above observations and tests suggest that punkies entering a room through a DDT-treated screen have several hours in which to seek out possible sources of a blood meal before they die.

A second formulation was prepared by mixing 2 parts of 20% DDT solution (heavy aromatic naphtha solvent); 1 part No. 10 lubricating oil, 1 part Lethane 60® (2-thiocyanoethyl laurate); and 4 parts kerosene.

The lubricating oil was added as a slow-drying sticker and the Lethane for more rapid knockdown. This formu-

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[2] The treated screens were kept in the test cartons and not exposed to additional weathering between tests.

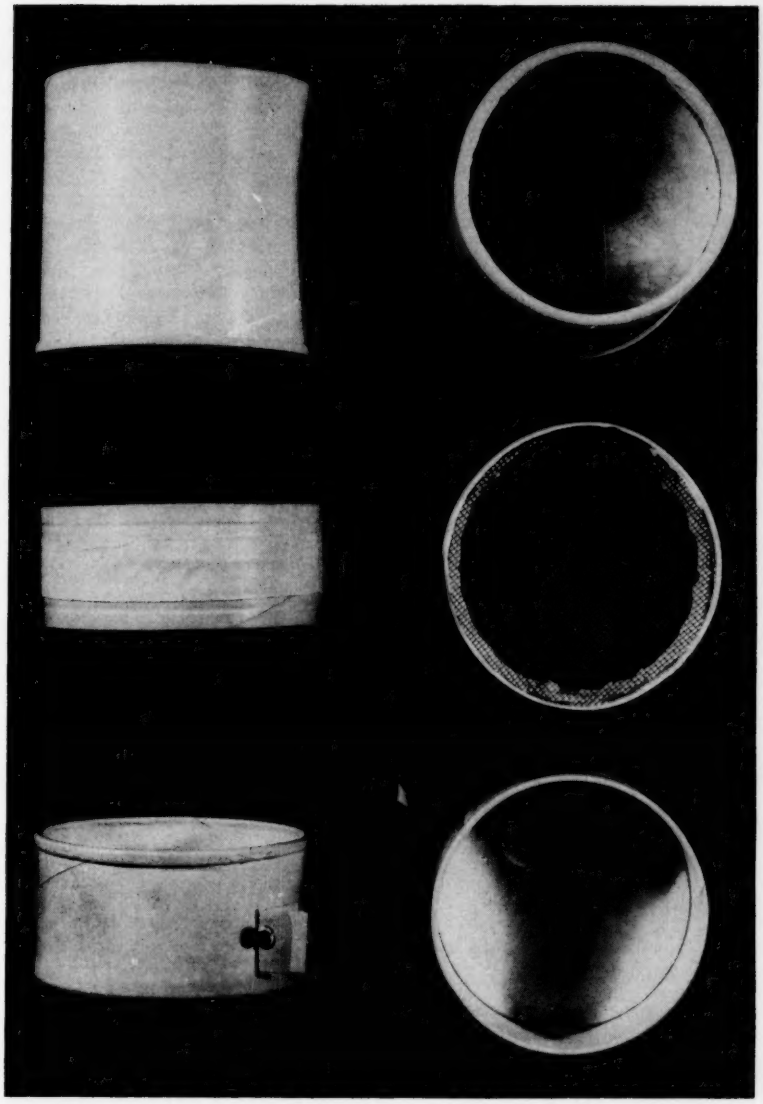

FIG. 1.—Two views of the components of test chambers used in evaluating the effectiveness of insecticide-painted screens as a control for punkies (*Culicoides obsoletus*).

lation was painted on a screen which was allowed to weather for 4 days before testing. On the fifth day, 20 punkies were placed in a carton with the treated screen; after 50 minutes all but three were stuck to it. When the screen was retested 9 days after being treated the punkies survived for longer periods and fewer stuck to the screen (table 1). Tests with this formulation were discontinued because dust, lint, silt and insects stick on the screen. This makes them unsightly and would reduce air movement and light if used in a dwelling (Trapido 1947).

A third formulation, consisting of 7.7% malathion (wt./wt.) dissolved in 80% ethyl alcohol, was prepared and painted on a screen. This has the advantage of leaving a nonsticky residue on the screen after drying. After 1 day, the painted screen was placed in a test carton and 20 punkies added. Three died within 30 minutes; 17 died within 35 minutes and all 20 punkies were dead within 45 minutes. During this period, only one of the punkies in the untreated screen check carton died.

The next day, another 20 punkies were placed in the treated screen carton. All were apparently normal 30 minutes later; after 45 minutes, 16 were dead or dying and within 60 minutes, all were dead. None of the 20 punkies in the untreated carton died during this period.

Table 1.—The effectiveness of chemically treated screens in killing punkies (*Culicoides obsoletus*).

Exposure in Hours and Minutes	Punkies Surviving (%)			
	10% DDT Solution	DDT-Lethane Lubricating Oil	7.7% Malathion-Alcohol Solution	Untreated
0:45	100	95	2	100
1:15	95	75	0	100
2:45	90	70	0	100
3:45	75	60	0	100
5:15	75	60	0	100
8:15	75	10	0	95
9:00	15	10	0	95
10:00	15	0	0	95
11:30	5	0	0	95
12:00	0	0	0	95
Number of punkies used per test				
	20	20	50	20
Days Between Treating Screens and Testing				
	16	9	3	—

The 7.7% malathion screen was again tested on the third day. The results are shown in table 1. Forty-nine out of fifty punkies were dead within 45 minutes and all were dead within an hour and 15 minutes.

The screen was tested again 13 days after being painted. Twenty punkies placed in the carton with 7.7% malathion-treated screen died within 45 minutes and only one out of 20 punkies in the untreated carton died during this period.

The screen was tested for the last time 21 days after it was treated with 7.7% malathion. In this instance 50 punkies were placed in the treated-screen carton and 20 in the untreated carton. Within 50 minutes all of the punkies in the treated-screen carton were dead while none died in the untreated check.

These tests indicate that 7.7% malathion-alcohol solution is superior to the 10% DDT solution and the DDT-lubricating oil-Lethane formulation tested in rapidly knocking down and killing punkies that pass through treated screens.

Although precise data on the effectiveness of the malathion formulation as a practical method of control in homes, especially the effects of weathering, were not obtained, several families that lived in especially high punkie nuisance areas reported that the formulation was very effective in reducing the annoyance in dwellings when painted on the screens.

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Reproducibility of the Toxicity Ratio of Allethrin to Pyrethrins Applied to House Flies by the Turntable Method[1]

W. A. Gersdorff, P. G. Piquett, Norman Mitlin, and Nathan Green
Entomology Research Division, Agric. Res. Serv., U.S.D.A.

Abstract

The 52 comparisons made in 10 years of the toxicity of allethrin and pyrethrins when applied by the turntable method to house flies (*Musca domestica* L.) were summarized. The ratio of toxicity of allethrin relative to pyrethrins at the 50% mortality level ranged from 2.23 to 3.61. A somewhat wider range was that due to the difference in slope of regression lines; the grand means of the ratios at the 5%, 50%, and 95% mortality levels were very close to two, three, and four times, respectively.

Since the first synthesis of allethrin in 1948 (Schechter *et al.* 1949), many extensive comparisons of its toxicity to house flies (*Musca domestica* L.) have been made relative to pyrethrins when the insecticides were applied by the turntable method. About half of the comparisons were made when the two were used as standards in the same study with other materials; the mortality data of most of these have been reported. Many were made in studies of new samples of the materials or as checks accompanying new chemical analyses of old samples after a period of use. Most of these have not been reported. Altogether, there were 52 comparisons, comprising 3,204 tests on 322,117 flies, representing 240 populations, with eight samples of allethrin and five of pyrethrins, over a period of 10 years. The purpose of the present study was to give all data similar treatment and report the final comparisons.

Samples.—The samples of pyrethrins comprised two extracted at this laboratory (P-1 and P-4) and three commercial products (P-2, P-3, and P-5). All except P-2 were known to be the complex contained in the extractives from pyrethrum flowers without further processing for the removal of materials other than the toxicants. The nature of P-2 (a 20%-concentrate) in this respect was unknown; however, it was dark olive green, an indication that it was not highly refined, and was frequently compared biologically with P-3 for stability. P-5 was received as the raw extract in hexane; this solvent was removed and the extractives dissolved in kerosene.

The analyses of the five pyrethrins samples, made in duplicate by the AOAC (Association Official Agricultural Chemists) method (1945–55), are given below. Stock solutions of them were prepared at concentrations of 18.3, 37.6, 21.4, 42.8, and 36.9 mg. of total pyrethrins per milliliter of refined kerosene (Deobase). Except when

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removed for the preparation of sprays, these solutions were kept in a dark cupboard at room temperature. At times they were reanalyzed. The samples of the insecticides were essentially stable chemically for several years when held at the concentrations of the stock solutions in the dark at room temperature. Four years was the maximum period of testing with the same sample.

Sample	Date Analyzed	Concentration (mg. per ml.)			Per Cent Type I of Total Pyrethrins
		Type I	Type II	Total	
P-1	March 1, 1945	—	—	20.0	—
	November 3, 1947	11.0	9.1	20.1	54.6
	July 2, 1948	10.2	8.1	18.3	55.7
	May 17, 1949	11.3	8.7	20.0	56.5
P-2	March 31, 1950	20.1	17.5	37.6	53.5
	June 5, 1951	20.4	19.2	39.6	51.5
	September 18, 1952	19.1	17.9	37.0	51.6
P-3	March 31, 1950	11.6	9.8	21.4	54.2
	June 5, 1951	10.1	9.4	19.5	51.8
	September 18, 1952	9.6	8.6	18.2	52.7
P-4	October 12, 1953	25.9	16.9	42.8	60.5
	May 8, 1955	28.2	18.8	47.0	60.0
P-5	April 13, 1955	20.0	16.9	36.9	54.2

The samples of allethrin included five preparations made in the Entomology Research Division (A-1, A-2, A-3, A-4, and A-7) and three produced by industrial companies (A-5, A-6 and A-8). All were prepared by the same procedure (Schechter *et al.* 1949) and all were purified. Samples A-2, A-7, and A-8 were ultimately distilled in a high vacuum, whereas the others were passed through a column of activated carbon to remove color and odor. Those samples prepared after a method of chemical analysis was developed were about equivalent in degree of purity; by the hydrogenolysis method, A-4, A-7, and A-8 were 94.0%, 94.4%, and 93.4% pure, respectively. All samples were about equivalent in toxicity; therefore, those with no chemical analysis were assumed to be as pure as the others. A stock solution of each was prepared at a concentration of 8 mg. per milliliter of kerosene. Since nothing is known regarding the toxicity of the contaminants, all concentrations of allethrin are given on the basis of sample weight. When the stock solutions were made, the samples were liquid, not having crystallized in part, as is possible (Schechter *et al.* 1951).

METHOD.—The method of comparison required the determination of dosage-mortality curves. Mortality of house flies caused by kerosene sprays at selected concentrations prepared from the stock solutions was determined in replicated tests made on the Campbell turntable (Campbell & Sullivan 1938). Knockdown of flies was also estimated, but since the results followed the pattern of tests previously reported, these measurements will not be given. Approximately 100 flies were used in each test. The average age of flies in a population fell in the range of 2 to 3½ days. All flies were from the National Association of Insecticide and Disinfectant Manufacturers' 1948 strain, a mixture made in the pupal stage from several colonies with no history of contact with an insecticide. They were reared in the laboratory by the same procedure, with only one difference of possible importance. Prior to September 1951 the larvae were almost always forced out of their culture jars with water; after that date they were allowed to pupate in the jars, and the pupae were separated from the dried medium by air suction.

To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to the maximum likelihood method of probit analysis as described by Finney (1952). From the regression equations the concentrations causing 5%, 50%, and 95% mortality were calculated and relative toxicity at each level estimated as the inverse ratio of the pertinent pair of concentrations. The median lethal concentrations (LC-50's) and the ratios at the three levels are given in table 1.

The mortality data not previously reported and the equations with other pertinent data for all comparisons are available to those requesting them.

Purified samples of allethrin still in a liquid condition are uniform, both chemically and biologically (Freeman 1955, Gersdorff & Piquett 1958a). The pyrethrins complex, however, may differ with different samples. The chemical analysis determines the proportion of pyrethrin I and cinerin I to the corresponding type II compounds, but does not give information on the proportion of the pyrethrins to the cinerins. The first proportion was about the same for four of the samples used, but for the other (P-4) it was appreciably higher and, therefore, the latter should be slightly more toxic (Gersdorff 1947). However, the effect of the small differences in chemical constitution of the pyrethrins on the ratio of toxicity is so slight that it is masked by the variability from other sources and may therefore be ignored. The weighted mean ratio, with the number of individual ratios indicated in parentheses, for each of the 13 samples is given below:

Sample	Allethrin	Pyrethrins
1	3.432 (1)	3.014 (2)
2	2.992 (2)	2.891 (13)
3	3.011 (1)	2.931 (13)
4	2.840 (10)	2.785 (13)
5	2.431 (1)	2.774 (11)
6	2.701 (1)	
7	2.837 (20)	
8	2.850 (16)	

Three of the authors separately performed both rearing and testing prior to July 1957; after that date the flies were reared by other workers. The weighted mean ratios obtained by the three were 3.01, 2.90, and 2.78, the last value being the mean of those comparisons including 11 of the 13 ratios based on the pyrethrins containing 60% pyrethrin I and cinerin I.

As estimated from the grand equations covering all comparisons, the toxicity of allethrin relative to pyrethrins, 2.90 times at the 50% mortality level, varies from twice at the 5% to four times at the 95% level. Had these three ratios been determined directly from the individual ratios given in table 1, weighted by any method or unweighted, their values would have differed only trivially. For example, with weights based on precision of measurement—reciprocals of the relative standard errors—the ratios are 2.11, 2.84, and 4.02, and with the unweighted method they are 2.11, 2.85, and 4.07. All are well within tolerance of the equational values. Therefore, it may be concluded that the toxicity of 93% to 95% pure allethrin as a space spray to house flies of a strain with no previous contact with an insecticide is, at the 50% mortality level, very close to three times that of a pyrethrins complex for which the percentage of pyrethrins I and cinerin I lies between 50% and 60% of the total pyrethrins; at the 5%

Table 1.—Toxicity to house flies of allethrin relative to pyrethrins.

Series	Month Tested	Samples A	Samples P	LC-50 (mg./dl.) Allethrin	LC-50 (mg./dl.) Pyrethrins	Toxicity Ratio at Indicated Mortality 5%	50%	95%
1948								
1[a]	Oct.	1	1	81.4±9.2	279.4 ± 9.0	2.86	3.43	4.12
1949								
2[a]	Mar.	2	1	78.9±3.5	223.4 ± 5.3	3.24	2.83	2.48
3	May	2	2	63.1±2.1	213.3 ±25.4	1.88	3.38	6.08
4	June	3	2	37.6±4.2	113.3 ± 6.4	2.21	3.01	4.10
5[b]	July	4	2	74.0±6.0	176.5 ±12.8	2.16	2.38	2.64
6	Aug.	4	2	73.1±1.9	181.0 ± 7.8	1.08	2.48	5.67
		5	—	74.5±2.1	—	1.16	2.43	5.09
7	Oct.	4	2	74.7±4.4	239.8 ± 5.4	3.25	3.21	3.16
1950								
8	Jan.	4	2	43.0±2.8	115.5 ± 6.9	1.36	2.69	5.37
		6	—	42.8±1.3	—	1.52	2.70	4.76
9	Feb.	4	2	93.1±4.7	310.3 ± 7.4	3.18	3.33	3.50
10	Mar.	4	2	96.7±1.7	268.6 ± 5.1	2.29	2.78	3.37
		—	3	—	267.4 ±16.7	1.97	2.77	3.89
11[c]	Apr.	4	3	65.7±4.8	167.0 ±12.0	2.28	2.54	2.83
12	Aug.	4	3	107.1±7.4	355.1 ± 8.0	3.44	3.32	3.20
1951								
13[d]	Jan.	7	3	82.8±1.8	254.0 ± 6.6	2.17	3.07	4.34
14[d]	May	7	3	94.9±2.5	219.5 ±20.4	2.28	2.31	2.34
15	June	7	2	78.8±7.6	241.0 ± 5.9	1.68	3.06	5.55
16[e]	July	7	2	78.2±5.4	282.5 ± 8.0	2.94	3.61	4.43
17	Oct.	4	3	85.6±5.5	229.8 ±16.9	1.55	2.69	4.64
1952								
18	Jan.	7	3	92.2±4.8	277.5 ± 4.7	2.95	3.01	3.07
19[e]	Feb.	7	3	78.7±3.4	259.3 ± 6.2	2.86	3.28	3.79
20[f]	Mar.	7	2	107.2±6.5	336.1 ±29.4	2.28	3.14	4.32
21[f]	Apr.	7	3	103.6±2.6	292.3 ±15.7	2.60	2.82	3.07
22[f]	Apr.	7	3	96.2±2.7	270.8 ±12.3	3.34	2.82	2.37
23[g]	May	7	3	107.9±2.6	326.1 ± 9.5	1.98	3.02	4.62
24[f]	June	7	3	91.1±7.0	230.0 ± 9.7	1.36	2.52	4.68
25[h]	July	7	3	77.1±1.6	242.4 ±13.0	2.65	3.14	3.72
1953								
26	Oct.	7	4	88.2±3.5	279.9 ± 7.6	1.62	3.17	6.23
27	Dec.	7	4	89.9±2.8	226.9 ± 5.5	1.64	2.52	3.89
1954								
28	May	7	4	101.1±3.9	265.8 ±13.4	1.71	2.63	4.05
29[i]	May	7	4	85.3±1.2	210.5 ±10.4	1.66	2.47	3.67
30	June	7	4	95.6±1.5	213.1 ±11.2	1.68	2.23	2.95
31[g]	July	7	4	87.2±3.7	220.0 ±10.3	1.59	2.52	4.02
32[j]	Aug.	7	4	93.4±1.5	226.2 ± 9.0	1.47	2.42	3.98
1955								
33	Mar.	7	4	67.8±3.0	201.9 ± 9.0	1.71	2.97	5.17
		8	—	64.1±2.4	—	1.83	3.14	5.37
34	Apr.	8	4	68.3±1.1	204.8 ± 7.7	1.67	3.00	5.39
		—	5	—	175.8 ± 9.6	1.48	2.57	4.49
35	May	8	4	71.8±1.3	192.3 ±10.9	1.77	2.68	4.06
36	June	8	4	67.1±1.4	228.8 ±12.9	2.56	3.41	4.55
1956								
37	Jan.	8	4	49.9±2.4	157.4 ± 4.0	1.88	3.16	5.28
1957								
38[l]	Jan.	8	5	75.8±3.2	188.2 ± 5.6	1.67	2.48	3.69
39[k]	Mar.	8	5	77.9±1.3	240.8 ± 4.6	2.56	3.09	3.73
40[l]	May	8	5	62.6±1.1	165.0 ± 5.7	1.76	2.64	3.95
41[k]	May	8	5	63.7±1.2	167.7 ± 5.8	1.72	2.63	4.04
42[m]	June	8	5	75.2±1.5	185.2 ± 3.9	2.11	2.46	2.86
43[k]	July	8	5	114.0±1.9	339.0 ±12.5	2.22	2.97	3.99
44[m]	Aug.	8	5	110.1±3.6	358.2 ± 7.7	2.33	3.25	4.55
45[l]	Oct.	8	5	105.5±4.4	300.9 ± 5.4	2.41	2.85	3.38
1958								
46[n]	May	8	5	110.9±3.8	299.7 ± 9.7	2.14	2.70	3.41
47[n]	Aug.	8	5	125.3±6.2	325.4 ±13.2	1.90	2.60	3.54
Grand				82.02±0.49	237.77± 1.49	2.13	2.899	3.94

[a] Gersdorff 1949.
[b] Gersdorff *et al.* 1951.
[c] Gersdorff *et al.* 1952.
[d] Gersdorff & Mitlin 1952.
[e] Gersdorff & Mitlin 1953.
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[m] Gersdorff & Piquett 1959b.
[n] Gersdorff & Piquett 1959c.

and 95% mortality levels the ratios are two and four times, respectively.

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The Toxicological Action of Three Organophosphorus Insecticides with Three Species of Mosquito Larvae[1]

CLAUDE H. SCHMIDT, and DONALD E. WEIDHAAS, *Entomology Research Division, Agric. Res. Serv. U.S.D.A.*

ABSTRACT

Toxicological studies were undertaken with radioactive-labeled parathion, Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate), and dimethoate to determine the dosage of these toxicants absorbed by larvae of *Anopheles quadrimaculatus* Say, *Aedes aegypti* (L.), *Aedes taeniorhynchus* (Wied.) The interrelationship of the dosage absorbed, the concentration, the time of exposure, and the mortality were also determined. Results indicated that the efficiency of uptake differed with the insecticide used. The concentration required to kill larvae did not necessarily reflect the amount of insecticide that entered the insect. For example, dimethoate in 24-hour exposure tests gave an LC-50 of 4.0 p.p.m. and an LD-50 of 0.0040 μg./larva; whereas Bayer 22408 gave an LC-50 of 0.009 p.p.m. and an LD-50 of 0.0020 μg./larva. The amount of insecticide found in larvae after 24 hours of exposure at the LC-50 concentration was only a very small percentage of the total to which they were exposed: with dimethoate, 0.02%; with parathion, 0.7%; and with Bayer 22408, 2.2%. Better methods of treatment could make some inferior toxicants into effective larvicides and should increase the efficiency of all toxicants. All three insecticides were excreted readily by larvae.

The relative effectiveness of insecticides against mosquito larvae is usually evaluated by comparing the mortalities when the insects are exposed in water containing different concentrations of the toxicants. Until radioactive tracers came into use no comparisons were based on the actual amount of material that entered the larvae.

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Since the determination of the toxicity of a chemical is influenced by penetration and type of exposure (Schmidt & Weidhaas 1958), studies are being made with several of the recently developed compounds having larvicidal action to show the interrelationship of concentration, exposure time, dosage absorbed, and mortality. All of these evaluations are conducted with radioactive compounds and new materials are included in the program as soon as tagged samples become available.

Furthermore, the growing resistance to insecticides from all over the world indicates that a more complete knowledge of how these materials act on mosquitoes would be most helpful. This is especially true since there is a world-wide attempt to eradicate malaria. The increased prevalence of resistance to chlorinated hydrocarbons, especially DDT and dieldrin (Brown 1958, Micks 1960), and experimental work showing that the organophosphorus materials can be used to control mosquitoes have focused attention on the toxicological action of these materials on mosquitoes.

Studies on the toxicological action of DDT, Co-Ral[1] (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), and dimethoate on larvae of *Anopheles quadrimaculatus* Say were reported by Schmidt & Weidhaas (1958), and additional data on the toxicological action of C^{14}-labeled DDT on larvae of *A. quadrimaculatus*, *Aedes taeniorhynchus* (Wied.), and *Aedes aegypti* (L.) were given by Weidhaas & Schmidt (1960). The present paper gives the results of additional tests with dimethoate and also with radioactive parathion and Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate).[2] All materials were labeled with phosphorus^{32} and their activity was 34 μc./mg. for parathion, 1.2 μc./mg. for Bayer 22408, and 14.2 μc./mg. for dimethoate. Three species of mosquitoes (*quadrimaculatus*, *taeniorhynchus*, and *aegypti*) were used with parathion. Since only a very limited amount of Bayer 22408 was available, this compound was tested only against *quadrimaculatus*. Dimethoate was used on both *quadrimaculatus* and *aegypti*.

PROCEDURE.—The standard Orlando test method was followed in which 25 fourth-instar larvae were exposed in 250 ml. of suspension or solution in a 400-ml. glass jar. Mortality readings were made at the desired intervals. The larvae were then removed by screening, washed with 250 ml. of distilled water, ground with a tissue grinder, plated, and radio-assayed in a PC-3 Model proportional gas-flow counter. The counting data were corrected for self-absorption and decay (Schmidt & Weidhaas 1958).

Larvae were first exposed to different concentrations of the labeled compounds for 24 hours to determine the dosage absorbed and the mortality. The dosage absorbed and mortality were then determined in tests in which both the exposure times and concentrations differed. In this manner the interrelationships of exposure time, concentration, dosage absorbed, and mortality were studied. Tests also were run to determine if larvae which had been exposed to 0.01 p.p.m. of parathion, 0.02 p.p.m. of Bayer 22408, or 5 p.p.m. of dimethoate for 2 to 5 hours excreted some of the insecticide when placed in clean water. Half of the insects were analyzed as soon as the exposure period had been completed, the others were transferred to clean water and analyzed 24 hours after the start of the test. All results are averages of two to three tests and are given in the tables.

Table 1.—Mortality and dosage absorbed with mosquito larvae exposed for 24 hours to various concentrations of P^{32}-labeled parathion.

Concentration (p.p.m.)	*A. quadrimaculatus*		*A. taeniorhynchus*		*A. aegypti*	
	Mortality (%)	Dosage Absorbed[a]	Mortality (%)	Dosage Absorbed[a]	Mortality (%)	Dosage Absorbed[a]
0.001	—	—	12	1.0	—	—
.002	0	2.0	—	—	0	1.5
.0025	—	—	18	2.6	—	—
.004	6	3.0	—	—	—	—
.005	—	—	78	4.0	52	3.4
.006	50	4.6	—	—	76	4.2
.007	—	—	—	—	82	4.3
.008	82	5.5	98	4.8	88	4.4
.009	—	—	—	—	96	4.8
.01	94	7.0	—	—	96	5.2

[a] μg./larva × 10^{-4}

RESULTS.—The mortality of larvae of *quadrimaculatus*, *taeniorhynchus*, and *aegypti* and the dosage absorbed in 24 hours of exposure to different concentrations of parathion are shown in table 1. The LC-50 and LD-50 are given below:

	LC-50	*LD-50* (μg./larva × 10^{-4})
A. quadrimaculatus	0.0060	4.6
A. taeniorhynchus	.0036	3.3
A. aegypti	.0048	3.5

The mortality of all three species was correlated with the concentrations used and the dosage absorbed. The absorbed dosage plotted against the concentration gave a straight-line relationship for *quadrimaculatus*, but not for *taeniorhynchus* or *aegypti*. With the latter two species when concentrations were increased the dosage absorbed decreased proportionately at the higher concentrations. Except for this fact the uptake of parathion was about the same for all three species. In previous tests (Weidhaas & Schmidt 1960) the uptake of DDT differed considerably from one species to another.

The mortality and absorbed dosages found in fourth-instar *aegypti* larvae after 24 hours of exposure to four concentrations of dimethoate are given in table 2. Plotted on a log-log scale, the dosage was proportional to the concentration, with a slope of about 1. Mortality occurred only at the higher concentrations, and the data were not sufficient for an accurate determination of the LC- or LD-50; however, the LC-50 was in the order of 4.6 p.p.m. and the LD-50 approximately 0.0064 μg./larva. At the LC-50 level, 25 larvae would contain only 0.16 μg. of the

Table 2.—Mortality and dosage absorbed with fourth-instar *Aedes aegypti* larvae exposed for 24 hours to four concentrations of P^{32}-labeled dimethoate.

Concentration (p.p.m.)	Mortality (per cent)	Dosage Absorbed (μg./larva × 10^{-4})
1	0	11
2	0	22
4	29	55
6	83	82

[1] The radiolabeled dimethoate was received from Lederle Laboratories, Division of the American Cyanamid Company and the radiolabeled Bayer 22408 from Robert Geary of the Vero Beach Laboratories. The parathion was purchased by the laboratory from the Radiochemical Center, Amersham, England.

Table 3.—Mortality and dosage absorbed with fourth-instar *Anopheles quadrimaculatus* larvae exposed for 24 hours to five concentrations of P^{32}-labeled Bayer 22408.

CONCENTRATION (P.P.M.)	MORTALITY (%)	DOSAGE ABSORBED (μG./LARVA$\times 10^{-4}$)
0.008	30	17
.01	67	25
.02	98	33
.03	100	43
.04	100	60

1,150 μg. present in the test jar, or about 0.01%. The small amount absorbed could explain the reason very high concentrations of this insecticide in water are required to kill the larvae. In the tests with Bayer 22408 shown in table 3, the mortality of *quadrimaculatus* larvae and the absorbed dosage both increased with the concentration. The estimated LC-50 and LD-50 were 0.009 p.p.m. and 0.0020 μg./larva, respectively. A plot of the absorbed dosage against the concentration on either an arithmetical or log-log scale showed a fair correlation between these two factors.

When the absorption of parathion was studied at different exposure times and concentrations, the mortality increased with the time of exposure at a given concentration or with the concentration at a given exposure time, as shown in table 4. When the dosage absorbed was plotted against the time of exposure at a given concentration and against the concentration at a given exposure time, the uptake over time or concentration was similar for *quadrimaculatus* and *taeniorhynchus*, but slightly less for *aegypti*. With *quadrimaculatus* a straight-line relationship on log-log paper was obtained for the dosage absorbed vs. time of exposure at all concentrations, and for the dosage absorbed vs. the concentration at 24 hours. With the four

Table 4.—Effect of different exposure times and insecticide concentrations on mortality and dosage absorbed.

INSECTICIDE	CONCENTRATION (P.P.M.)	EXPOSURE (HOURS)	MORTALITY (%)	DOSAGE ABSORBED (μG./LARVA$\times 10^{-4}$)
		Anopheles quadrimaculatus		
Parathion	0.005	2	0	1.2
		5	0	2.1
		8	0	2.5
		19	29	3.7
		24	73	4.4
	.006	2	0	1.8
		5	0	2.8
		8	0	3.5
		19	43	5.2
		24	84	5.1
	.009	2	0	2.5
		5	0	3.1
		8	0	4.5
		19	95	7.1
		24	100	6.4
Bayer 22408	0.008	2	0	4
		5	0	15
		8	2	12
		19	5	16
		24	18	14
	.01	2	0	5
		5	0	12
		8	1	18
		19	54	20
		24	93	21
	.02	2	0	15
		5	0	17
		8	2	22
		19	47	24
		24	96	20
Dimethoate	2.0	2	0	5
		5	0	10
		8	0	17
		19	7	32
		24	28	34
	3.5	2	0	9
		5	0	21
		8	0	30
		19	40	58
		24	79	68
	5.0	2	0	15
		5	0	31
		8	0	45
		19	71	84
		24	93	84
		Aedes taeniorhynchus		
Parathion	0.0025	2	0	.7
		5	0	1.2
		8	0	1.8
		19	1	2.6
		24	5	3.5
	.004	2	0	.9
		5	0	1.7
		8	1	2.7
		19	1	4.1
		24	20	4.7
	.008	2	0	1.7
		5	0	3.4
		8	0	4.6
		19	49	6.8
		Aedes aegypti		
Parathion	0.003	2	0	.2
		5	0	.6
		8	0	1.1
		19	0	1.9
		24	0	2.1
	.005	2	0	.5
		5	0	1.2
		8	0	1.6
		19	7	2.8
		24	24	2.9
	.008	2	0	1.1
		5	0	1.7
		8	4	2.7
		19	55	3.8
		24	91	4.0

other exposure periods, the dosage absorbed vs. the concentration did not give a straight-line relationship. In previous tests with DDT the reverse was true, *i.e.*, the dosage absorbed vs. time of exposure did not give a straight line whereas the dosage absorbed vs. the concentration did. Whenever a straight line was not obtained, the curve flattened. One reason for this difference is that parathion is presumably equally distributed in test jars, but as Bowman *et al.* (1959) showed, DDT is concentrated in the water interface or is lost from test jars by codistillation with water vapor. Loss by codistillation would account for the decrease in uptake over time and loss by codistillation plus concentration in the interface are responsible for the difference in uptake between species.

The mortalities and absorbed doses in fourth-instar *quadrimaculatus* larvae after exposure to three concentrations of Bayer 22408 and dimethoate for different periods of time also are given in table 4.

With both compounds, mortality increased with time of exposure at a given concentration or with the concentration at a given exposure time except with the 19-hour exposures at 0.01 to 0.02 p.p.m. of Bayer 22408. With dimethoate the dosage absorbed increased proportionately with the concentration when time was constant, and with time when the concentration was constant. Mortality data were not sufficient for precise correlation with the time of exposure or with dosage absorbed. However, the LD-50 (μg./larva) for 19 hours' exposure was 1.5 times as great as that for 24 hours, since the increase in mortality from 19 to 24 hours at a given concentration was much greater than the increase in the dosage absorbed. With Bayer 22408 the dosage absorbed was not proportional to the concentration except with the 2-hour exposure. At other exposure times, the ratio of the absorbed dosage to the concentration was about equal at 0.008 and 0.01 p.p.m., but much lower at 0.02 p.p.m. When the dosage absorbed was plotted against the time of exposure at a given concentration, the rate of uptake per unit of time decreased as the time of exposure increased.

As shown in table 5, all three of the compounds were excreted. Based on the 5-hour exposure tests against *quadrimaculatus* the elimination of dimethoate was quicker and parathion slower than for Bayer 22408. *A. quadrimaculatus* excreted less parathion than either *taeniorhynchus* or *aegypti*. The dimethoate and Bayer 22408 were lost by *A. quadrimaculatus* in larger quantities than the parathion by the two *Aedes* species. The parathion losses after 24 hours were similar for all three species following the 2-hour exposure. *A. taeniorhynchus* larvae exposed to 0.01 p.p.m. of parathion for 5 hours absorbed a little more than twice as much of the insecticide but excreted three times more than those larvae exposed to the same amount for 2 hours.

Table 5.—Effect of varying exposure time on the dosage absorbed, 24-hour mortality, and excretion for larvae exposed 2 to 5 hours to 0.01 p.p.m. of parathion, 0.02 p.p.m. of Bayer 22408, or 5 p.p.m. of dimethoate.

Insecticide	Exposure (hours)	Dosage Absorbed (μg./larva ×10⁻³)	Excretion Loss		Mortality (%)
			Per Cent	Rate (μg./larva/hr. ×10⁻³)	
Anopheles quadrimaculatus					
Parathion	2	1.8	39	3.2	3
	5	3.1	23	3.7	13
Bayer 22408	3	17	59	48	53
	5	17	53	47	53
Dimethoate	5	28	64	95	5
Aedes taeniorhynchus					
Parathion	2	2.4	33	3.6	0
	5	5.1	40	11.0	0
Aedes aegypti					
Parathion	2	1.9	37	3.3	0
	5	2.4	50	6.3	4

DISCUSSION.—Over the past 2 years, five labeled insecticides have been tested to study the relationship of the concentration, the absorbed dose, and the mortality. A summary of the data reported previously (Schmidt & Weidhaas 1958, Weidhaas & Schmidt 1960) and in this paper on the results of 24-hour exposures of *Anopheles quadrimaculatus* larvae is presented below:

LC-50		*LD-50*		*Per Cent of Insecticide Removed from Solution*	
Insecticide	*p.p.m.*	*Insecticide*	*μg./larva*		
Parathion	0.0060	Parathion	0.00046	Dimethoate	0.02
DDT	.0060	Bayer 22408	.002	Parathion	.7
Bayer 22408	.009	Co-Ral	.004	Co-Ral	1.0
Co-Ral	.04	Dimethoate	.004	Bayer 22408	2.2
Dimethoate	4.0	DDT	.006	DDT	10.0

On the basis of the LC-50's parathion and DDT appeared to be the most toxic; Bayer 22408 and Co-Ral were intermediate; dimethoate was the least toxic and required 666 times the concentration of parathion or DDT. On the basis of the dosage absorbed, parathion appeared to be 13 times as toxic as DDT, 8.7 times as toxic as Co-Ral or dimethoate, and 4.3 times as toxic as Bayer 22408. These results indicated that the efficiency of uptake differs with different chemicals, a conclusion that is further borne out by the percentage of the available insecticide removed by the larvae. The concentrations required to kill did not necessarily reflect the amount of insecticide that entered the insect. If some better method of getting dimethoate, for example, into the insect can be found it could be about as effective as any of the materials tested except parathion. With most of the compounds studied so far the percentage of the toxicant found in the larvae has been low. Better methods of treatment are needed.

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Treated Bags for Control of the Corn Earworm and Fall Armyworm[1]

C. C. Blickenstaff[2] and L. F. Bauman[3]

Abstract

Shoot and tassel bags used in hand pollination of field corn inbreds and single crosses were treated with insecticides to test their effectiveness in preventing damage by larvae of the corn earworm (*Heliothis zea* (Boddie)) and fall armyworm (*Laphygma frugiperda* (J. E. Smith)). Materials tested in descending order of effectiveness were: aldrin, heptachlor, dieldrin, chlordane, DDT, and toxaphene. Shoot bags coated on the inside with 1% concentrations of aldrin or heptachlor, or with their open ends dipped in 5% concentrations prevented ear damage when left on the ears following hand pollination. The use of treated bags on garden sweet corn appeared to have little practical value.

It was generally observed in southern Georgia that the presence of either shoot or tassel bags covering the developing ears of corn did not protect them from larvae of the corn earworm (*Heliothis zea* (Boddie)) or the fall armyworm (*Laphygma frugiperda* (J. E. Smith)). Sometimes the presence of the bags appeared to increase damage. This result is of particular importance to corn breeders concerned with seed increase of inbred lines or with securing crosses between inbreds, because of the small ears and relatively few seeds produced per ear.

Dahms *et al.* (1951) reported the use of aldrin-treated bags for protecting sorghum heads from corn earworms and corn leaf aphids (*Rhopalosiphum maidis* (Fitch)), and later (1955) the methods and materials used in treating bags. Bags impregnated with DDT, heptachlor, aldrin, dieldrin, lindane, and a commercial mixture of methyl parathion and parathion gave effective earworm control. Lindane, aldrin, and heptachlor also gave satisfactory control of the corn leaf aphid. Lindane, however, caused a high percentage of sterile florets. Bags streaked on the inside with aldrin gave better control than those impregnated with the insecticide at the same dosage rate per bag.

Methods and Materials.—In the process of hand pollination in corn-breeding programs, the developing ears are covered by small shoot bags until the silks have emerged and are ready for pollination. At the time of pollination, shoot bags may be discarded entirely, torn off at the top and then crinkled over, or removed temporarily and then replaced intact. The pollinated ears are then covered with larger tassel bags. The difference in procedure is influenced partly by the kind of crosses being made, but mostly by personal choice of the breeder. Tassel bags are used also for enclosing and collecting pollen from tassels and transferring it to the silks.

Several tests were made at Tifton during 1955, 56, and 57 to evaluate the effectiveness of treated bags in protecting ears of corn from corn earworm and fall armyworm damage. Four tests involved single crosses of field corn and one a field-corn inbred. Four tests were made on sweet corn since it was considered that the protection of home garden plantings might be practical providing pollination could be effected naturally. Shoot bags, except as otherwise noted, were made of glassine and measured $2\times1\times7$ inches. Tassel bags were made of brown Kraft paper sealed with waterproof glue and measured $7\frac{1}{2}\times5\times15\frac{1}{2}$ inches. Bags were coated with insecticide emulsions to cover the entire inner and outer surfaces, the inner surface only, or the surfaces around the opening for a distance of approximately 1 inch. The coating was done by dipping or pouring the insecticide on the bags and then standing them on end to drain in hardware cloth baskets in front of a fan. Many shoot bags could be treated around the open end in a short time. Only 1 gallon of 25% aldrin diluted to 5% is required to treat 60,000 bags in this manner. Various combinations of treated shoot and tassel bags were used on field corn, with shoot bags either discarded or left on the ears intact or with their ends torn off and crinkled over at pollination. Shoot bags were placed intact on sweet corn ears at different times in relation to silking, or bags ventilated in different ways were placed on the ears before silking. At harvest each ear was examined for damaged kernels.

Table 1.—Effectiveness of shoot bags completely dipped in 5% concentrations of several insecticides and handled in two ways at pollination against the corn earworm and fall armyworm on the field corn single-cross 2-07×0-708.

Insecticide	Condition of Shoot Bag at Pollination	Ears		Kernels	
		Infested (%)	Control (%)	No. Damaged per Ear	Control (%)
Aldrin	Crinkled	10	89.2	2.5	97.2
	Intact	6	93.5	0.5	99.4
Heptachlor	Crinkled	18	80.6	3.1	96.6
	Intact	21	77.4	1.9	97.8
Chlordane	Crinkled	43	53.8	6.2	93.1
	Intact	31	66.7	8.9	90.1
DDT	Crinkled	50	46.2	21.1	76.5
	Intact	36	61.3	12.2	86.4
Check (no bags)		93	—	89.9	—

Tests on Field Corn.—In the first test shoot bags were completely dipped in 5% concentrations of insecticides, applied to ear shoots of the single cross F44×F6, and left on the ears after pollination. This early corn was subjected to moderate corn earworm and light fall armyworm infestations. Of 50 ears examined for each treatment, the per cent of control based on infested ears was as follows: Aldrin 100, heptachlor 100, chlordane 95, DDT 93, and toxaphene 89. Of the ears covered with untreated bags 84% were infested.

A second test was conducted on a late planting of the single-cross 2-07×0-708 subjected to heavy infestations of both insects. Shoot bags were treated with aldrin, heptachlor, chlordane, and DDT at the same rate and in the same manner as the first test. From 14 to 21 ears, with an average of 18, were used per treatment. The data in table 1 indicate that aldrin-treated shoot bags gave the most effective ear protection, with an average control of

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[2] Entomology Research Division, Agric. Res. Serv., U.S.D.A.

[3] Formerly Crops Research Division, Agric. Res. Serv., U.S.D.A.

Table 2.—Effectiveness of two insecticides applied to shoot bags in two ways at varying concentrations in controlling the corn earworm on the field corn single-cross GT 112×L578.

Method of Treatment, Insecticide Concentration (%) and Average Dosage per Bag (mg.)		Ears		Kernels	
		Infested (%)	Control (%)	Damaged (%)	Control (%)
		Heptachlor			
Coated inside	0.25	3.7	9	86.1	1.2	88.6
	0.50	6.9	7	89.2	2.0	81.0
	1.00	14.1	9	86.1	0.9	91.4
Open end dipped	1.25	3.0	17	73.8	1.5	85.7
	2.50	6.8	22	66.1	3.7	64.8
	5.00	13.0	2	96.9	0.4	96.2
		Aldrin			
Coated inside	0.25	4.2	26	60.0	3.2	69.5
	0.50	8.2	17	73.8	2.9	72.4
	1.00	15.2	9	86.1	1.4	86.7
Open end dipped	1.25	5.5	40	38.5	2.7	74.3
	2.50	11.6	40	38.5	2.6	75.2
	5.00	23.5	8	87.7	1.0	90.5
		Untreated			
	—	—	65	—	10.5	—

Table 3.—Effectiveness of combinations of shoot and tassel bags treated at the open end by dipping in different concentrations of aldrin for corn earworm control.

Per Cent of Aldrin Applied to		Ears		Kernels	
Tassel Bags	Shoot Bags[a]	Infested (%)	Control (%)	Damaged (%)	Control (%)
		Single Cross GT112×L578			
1.25	1.25	43	37.7	5.4	41.9
1.25	5.0	58	13.0	4.1	55.9
2.5	2.5	39	43.5	3.1	66.7
2.5	5.0	48	30.4	3.6	61.3
5.0	0	28	59.4	1.5	83.8
5.0	5.0	42	39.1	2.5	73.1
Untreated Bags		69	—	9.3	—
		Inbred GT112			
5.0	0	49	41.7	8.9	61.8
5.0	5.0	48	42.9	8.9	61.8
0	5.0	77	8.3	22.4	3.9
Untreated Bags		84	—	23.3	—

[a] Discarded at time of pollination.

91.4% in terms of ear infestation and 98.3% in terms of kernels damaged. Leaving the shoot bags intact at the time of pollination usually gave better protection than tearing off the closed end, pollinating through it, and then crinkling it over. In the same test and on comparable ears, the use of untreated shoot bags discarded at pollination and replaced with DDT-treated tassel bags resulted in 48.4% control in terms of infested ears and 83.5% in terms of kernels damaged. Though this method gave protection nearly equal to that of DDT-treated shoot bags, more than 17 times as much insecticide was needed to treat the tassel bags. Where untreated shoot bags were left on the ears at pollination and covered with DDT-treated tassel bags, ear infestation and kernel damage increased over that of open-pollinated ears by about 5% and 23%, respectively.

In a third test shoot bags were either coated on the inside or dipped at their open ends. Shoot bags were replaced on the ears following pollination. The test was conducted on the single-cross GT112×L578, which is susceptible to the corn earworm, and the test plants were subjected to a light infestation of the pest. From 40 to 53 ears were used per treatment. Heptachlor was slightly more effective than aldrin. For each insecticide, bags coated inside with 1% concentrations were slightly less effective than those with their open ends dipped in the 5% concentration. Bags treated at the higher dosage rates gave 86% to 97% control in terms of both infestation and numbers of kernels damaged. The data are given in table 2.

A fourth test was conducted on the same single-cross hybrid as in the preceding one. Both shoot and tassel bags were treated by dipping 1 inch of their open ends in different concentrations of aldrin. Tassel bags absorbed seven times as much liquid as shoot bags for comparable treatments. Shoot bags were discarded at the time of pollination, and tassel bags remained over the ears until harvest. From 50 to 60 ears were used per treatment. The data are given in table 3. No treatment gave adequate control. The higher the insecticide concentration on tassel bags the greater was the protection obtained, but increasing the concentration of insecticide on shoot bags did not result in increased protection. This was probably because of the short time that shoot bags were present on the ears. Average control for the tassel bags treated with 5% concentrations was 49.2% in terms of infested ears and 78.5% in damaged kernels.

In a fifth test, shoot and tassel bags treated by dipping the open ends in 5% aldrin were applied to ears of the inbred GT112. Shoot bags were discarded at pollination, when tassel bags were placed over the ears. From 53 to 63 ears were used per treatment. In general these data, also summarized in table 3, agree with those obtained for similar treatments on GT112×L578. The shoot bags had little effect on control. Treated tassel bags gave an average control of 42.3% in terms of infested ears and 61.8% in damaged kernels.

In 1956 shoot bags treated by dipping their open ends in 5% aldrin were used on a large scale on late-planted inbred lines in a nursery located at Tifton, Ga. The bags were replaced intact on the ears after pollination. In spite of a heavy natural infestation of corn earworm larvae, the bagged and treated ears were effectively protected.

TESTS ON SWEET CORN.—In the first test on sweet corn an attempt was made to obtain corn earworm control with treated shoot bags while at the same time allowing pollination to occur naturally by ventilating the upper portion of the bags. If effective, this method would require but one control operation in market sweet corn production or the home garden. Shoot bags were treated with aldrin at 2.5% and 5% concentrations by (1) dipping the bottom 2 inches, (2) dipping the entire bag, or (3) coating the inside. Bags were ventilated by cutting the closed upper ends into strips or by punching openings of different numbers and shapes through them as illustrated in figure 1. These bags were placed on ears of an early planting of Golden Cross Bantam which had suffered from drought at pollination. Only those plants that were normal and silked within a 5-day period were used. The number of ears per treatment varied from 14 to 24, with an average of 18.4. The data are summarized in table 4. Bags treated with 5% aldrin gave better control than those treated with 2.5%; those completely dipped gave almost twice the protection as those having only the bottom 2 inches dipped, and about 10% more protection than those

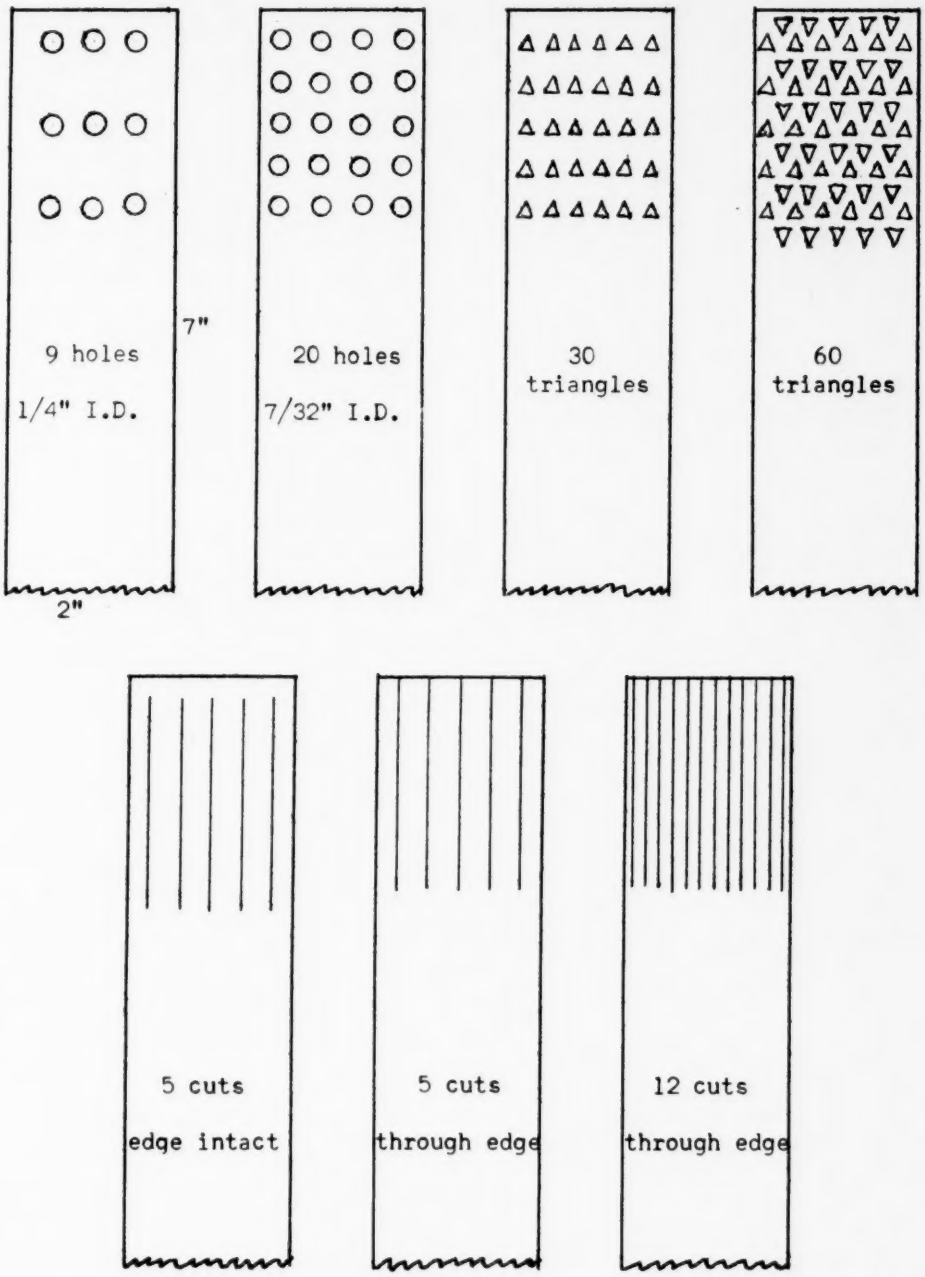

FIG. 1.—Methods of ventilating treated shoot bags to determine effect on corn earworm control and pollination.

treated on the inside only. Pollination was greatly reduced by placing ventilated bags over ears before silking. Placing entire bags over ears after full silk emergence did not reduce pollination and still gave from 48% to 73% earworm control.

The second test compared the effectiveness of bags treated with aldrin, heptachlor, or dieldrin by coating their insides with 1% concentrations of the insecticides or dipping 2 inches of their open ends in 5% concentrations, and applying the bags to ear shoots at various intervals

Table 4.—Effect of aldrin treatment and shoot bag ventilation on corn earworm control and pollination of sweet corn ears.

Shoot Bag Placement and Method of Ventilation	2.5% Aldrin			5% Aldrin		
	Ears Infested (%)	Control (%)	Pollination Rating[a]	Ears Infested (%)	Control (%)	Pollination Rating
			Bottom 2 Inches Treated			
Bags placed before silking:						
5 cuts edge intact	68	20.0	2.1	42	50.6	2.1
5 cuts through edge	72	15.3	1.7	53	37.6	1.3
12 cuts through edge	62	27.0	1.5	39	54.1	1.4
Means	67	21.2	1.8	45	47.1	1.6
			Entire Bag Treated			
5 cuts edge intact	44	48.2	1.4	14	83.5	1.8
5 cuts through edge	37	56.5	1.5	25	70.6	1.7
12 cuts through edge	25	70.6	1.4	45	47.1	1.6
Means	35	58.8	1.4	28	67.1	1.7
9 holes $\frac{1}{4}$ inch	—	—	—	33	61.2	1.5
20 holes $\frac{7}{32}$ inch	16	81.2	1.9	35	58.8	2.0
30 triangles	—	—	—	50	41.2	1.8
60 triangles	—	—	—	40	52.9	1.8
Bags placed after silking:						
No ventilation	35	58.8	2.4	23	72.9	2.5
			Inside of Bag Treated			
No ventilation	44	48.2	2.6	33	61.2	2.7

Untreated check (no bags): 85% of ears infested; 2.5 pollination rating

[a] Pollination rating: 1 (poor) to 3 (good).

after full silk emergence. An early planting of Iochief grown under nearly ideal weather conditions was used. Ears were usually considered in full silk on the second day after the first silk appearance. The number of ears per treatment averaged 45. The data are summarized in table 5. For all treatments of aldrin and one with dieldrin, bags treated on the inside gave significantly more effective control than those with their ends dipped. Significantly more effective control was obtained by applying bags on the day of or 1 day after full silk than at 4 days. The most control was obtained with bags treated with an inside coating of aldrin used on the day of or the day after full silk. Of the treatments applied 4 days after full silk, only those employing the inside coating with aldrin and heptachlor differed significantly from the check. Aldrin and heptachlor were nearly equal in performance after 2 and 4 days, but dieldrin was less effective. Pollination was reduced, as shown by the loss in length of ear covered by kernels, in all but the 4-day application.

A third test compared three types of bags treated with 5% aldrin by dipping their open ends in the insecticide. The glassine bags, applied to ears 2 days following full silk, measured $2\frac{1}{2}\times7\frac{1}{2}$ inches without side folds, 3×8 inches without side folds, or $2\times7\frac{1}{2}$ inches with $\frac{1}{2}$-inch side folds. Forty-five ears were used for each treatment. The average percentages of infested ears harvested from the three different-sized bags were 50.6, 45.0, and 46.6, respectively. Untreated ears averaged 83.6%.

A fourth test compared the effectiveness of bags in the presence or absence of flag leaves. Sweet corn varieties differ greatly in the extent to which the ear shucks develop at their tips into flag leaves, similar to but smaller than stalk leaves. The presence of many well-developed flag leaves would tend to hold a covering bag away from the shucks themselves. Bags coated on the inside with 1% aldrin were applied 2 days after full silk to ears of Golden Cross Bantam. On alternate rows the flag leaves were clipped off just before bags were put on. Forty-five ears

Table 5.—Effect of treated shoot bags on corn earworm infestation and seed set when applied to sweet corn ears with relation to time of silking.

Days After Full Silk on Which Bags Were Applied	Per Cent of Ears Infested Using Bags Treated With:						Average Length of Ear Covered by Kernels (in.)	Loss in Kernel-Ear Length (%)
	Aldrin		Heptachlor		Dieldrin			
	Inside[a]	Dipped[b]	Inside	Dipped	Inside	Dipped		
0	8.5	34.3	—	—	—	—	5.98	19.6
1	4.5	27.2	—	—	—	—	6.54	12.1
2	20.8	40.3	31.9	27.0	44.6	45.9	6.98	6.2
4	43.9	60.7	46.2	55.7	55.0	72.5	7.50	0.0
Check (unbagged ears)			74.4				7.44	
L.S.D. @ 5% level			19.6					

[a] Bags coated on the inside surface with 1% concentration.
[b] Bags dipped at the open end in 5% concentration.

were used for each treatment. The average infestation of ears with flag leaves removed was 36.5% and with flag leaves present 33.4%.

DISCUSSION.—Aldrin was the most effective of the insecticides used in the treatment of shoot bags against both the corn earworm and the fall armyworm. Heptachlor, dieldrin, chlordane, DDT, and toxaphene were somewhat less effective in the order listed.

Shoot bags treated with 5% concentrations at their open ends were somewhat more effective than those coated with 1% concentrations on the inside and were much easier and simpler to prepare. Discarding treated shoot bags at the time of pollination and relying only on treated tassel bags for protection against infestation until harvest did not give adequate protection. Much better control was obtained by leaving the shoot bags on the ears after pollination; tearing off the tops of the bags and crinkling them over slightly decreased the degree of control. The large-scale application of treated shoot bags to late-planted inbred lines in a nursery gave satisfactory control.

On sweet corn, no method was found of perforating treated bags which would allow natural pollination to occur satisfactorily. However, the methods described might be of practical value to the breeder of either field or sweet corn, especially where corn earworm or fall armyworm infestations are a problem.

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Studies of the Eggs of a Strain of Two-Spotted Spider Mite, *Tetranychus telarius*, Resistant to Parathion[1]

JALIL K. ABUL-HAB[2] and E. M. STAFFORD[3]

ABSTRACT

A resistant strain of the two-spotted spider mite, *Tetranychus telarius* (L.), was developed by exposing the active stages to parathion residues on bean leaves. Resistance of adult females was also developed by selective pressure on eggs and immature stages only. Eggs of the resistant strain were resistant to parathion in all stages of development. Within the resistant and nonresistant strains there were no significant differences in response to parathion from eggs in various stages of development.

With eggs ⅓ to ⅔ through the incubation period, malathion was ineffective as an ovicide and there was little difference in the response of the parathion-resistant and nonresistant strains. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate), Tedion® (*p*-chlorophenyl 2,4,5-trichlorophenyl sulfone), and Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol) were more effective ovicides. The parathion-resistant eggs required only from 4 to 6.7 times as much of these three insecticides as eggs of the nonresistant strain at the LD_{50} level. With parathion the LD_{50} for the eggs of the resistant strain was 278 times the LD_{50} for the nonresistant strain.

Esterase activity on indophenyl acetate appeared early in the incubation period of the eggs. Activity up to 8 hours after eggs were laid was significantly greater in the resistant strain than in eggs of the nonresistant strain.

A search in the literature revealed few records of the response of the eggs of insecticide-resistant arthropods to ovicides (Cole 1955, Riehl *et al.* 1959). Furthermore, no report has been found which deals with the response of the eggs of resistant arthropods to treatments with ovicides at various stages of their embryonic development. However, this subject has been studied with eggs of non-resistant insects (Lord & Potter 1951, Salkeld & Potter 1953, Potter *et al.* 1957, Smith & Wagenknecht 1959). The present study was undertaken to investigate the possible extension of parathion resistance from the adult females to the eggs of the two-spotted spider mite, *Tetranychus telarius* (L.). In addition, the cross-tolerance of such eggs to acaricides chemically related and not related to parathion, and the response to parathion of eggs at different stages of development were to be considered. Also to be considered was esterase activity of egg homogenates on the substrate, indophenyl acetate.

MATERIALS AND METHODS.—Throughout the study the parathion-resistant and nonresistant strains of *T. telarius* were isolated on plants in cages covered with fine mesh nylon. The cages rested on wet sand in a tray that was placed in a larger tray to provide a water barrier about 10 inches wide on all sides. This isolation method was developed by Morishita (1956). A colony of mites was split into two colonies, one of which was subjected to the selective pressure of parathion to build up resistance. The other colony was maintained without exposure to any chemical. Young and vigorous plants of Henderson's bush lima beans were used as food throughout. The soil and the pots were always sterilized in live steam and stored for later use. All of the rearing work was carried out in the greenhouse.

The selection process was accomplished by dipping the infested plants in increasing concentrations of parathion emulsions. Triton X-100 at a concentration of 0.1% was used as an emulsifing agent. When the plants were dry the treated leaves were placed on new plants for 2 days so that the surviving individuals would move from the old, dry plants to the new plants. The survival rates at each treatment with parathion were determined from predipping and postdipping counts of mites on the lower surfaces of 25 infested leaves. The leaves were maintained on wet cotton in petri dishes. Twenty-four hours after dipping, the surviving mites on the leaves were counted and recorded.

The selection process extended for 16 months and

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[2] University of Baghdad, Iraq.

[3] Department of Entomology, Davis.

covered some 35 generations of the mites. The dippings were made at intervals of 15 to 37 days, depending upon the time required for the population of the surviving individuals to build up to sufficient levels for further tests. This time, in turn, depended in part upon the temperature prevailing in the greenhouse. Preliminary observations showed that the mites needed about 10.5 days to complete one generation when the temperature was between 65° F. and 95° F. and 18 days when the temperature was between 65° F. and 76° F.

The selective pressure was applied in such a way that immature and mature motile stages of the population were exposed to the same concentration of parathion for four successive times. Then the concentration of parathion was increased and the process was repeated. The initial concentration of parathion was 0.02% and the final concentration was 0.2%, a ten-fold increase. The step-like increase in the concentration of parathion allowed a survival rate of 3% to 15% only.

The susceptibility of the adult females or motile immature stages of both normal and selected mites was determined by exposure to residues of parathion. Young and vigorous leaves were placed, lower surfaces up, in petri dishes prepared as described above. These leaves were sprayed with different concentrations of parathion emulsion with a DeVilbiss No. 15 adjustable atomizer. The mites were transferred to the leaves after the surface dried. Thirty to thirty-five adult females were transferred individually to each leaf using a camel's-hair brush. In tests with immature stages, the mites were transferred by placing a small piece of an infested leaf on each sprayed leaf for 8 hours. This period of time was enough for the source leaf pieces to wither and for the 30 to 40 young mites to move over to the treated leaves.

Mortality was checked 24 hours after the mites had been introduced to the parathion residues on the leaves. The total number of mites in a replicate was taken as the sum of living, moribund, and dead mites actually found on the leaves. The individuals which had wandered off the leaves and died on the wet cotton were disregarded. This method of determining mortality was found to be better than the use of a sticky barrier around the mites many of which wandered into the barrier with resulting high mortality. Moribund mites were counted as dead. The tests for the detection of resistance to parathion in the female mites were repeated on three different dates. After the first test the parathion treatments were reduced from an average of one every 24 days to an average of one every 114 days. After the second test treatment was discontinued altogether.

In contrast to the treatments of the motile stages, direct sprays were used in the treatments of the eggs. The eggs were treated at nine different stages of embryonic development. Since the average incubation period of the eggs varied with the season when the spraying was done, preliminary tests to determine the incubation period were made prior to each treatment. The responses of the eggs were determined at the following age groups expressed as fractions of the average incubation period: 0–1/3, 1/3–2/3, 2/3–1, 0–1/9, 1/9–2/9, 2/9–1/3, 2/3–7/9, 7/9–8/9, 8/9–1.

Eggs of the different age groups were secured by transferring females to clean leaves placed on prepared and numbered petri dishes. The females were allowed to lay eggs for a period equal to one-third of the predetermined incubation period. Then the females were removed to a second batch of clean leaves while the eggs were counted, recorded, and set aside under protective cages in the greenhouse. After another period equal to one-third the total incubation period the females were again removed to a third batch of clean leaves and their eggs were counted, recorded, and set aside. After a third period of the same duration, the females were destroyed and the eggs were counted and recorded. At this time the three batches of eggs were from $\frac{2}{3}$ to fully developed, from $\frac{1}{3}$ to $\frac{2}{3}$ developed, and from zero to $\frac{1}{3}$ developed. The eggs were sprayed immediately after those of the third batch were recorded. Survival counts were made after 6 to 12 days, depending upon the length of the incubation period at the time of spraying. Eggs of the other age groups listed were obtained in a manner similar to that given above.

Tests were carried out to detect cross-tolerance of eggs to Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate), malathion, Tedion® (*p*-chlorophenyl 2,4,5-trichlorophenyl sulfone), and Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol). The eggs were sprayed when they were $\frac{1}{3}$ to $\frac{2}{3}$ developed. Otherwise, the procedure of exposing the eggs was the same as described above.

The LD_{50} and the LD_{95} were determined by the probit analysis method of Litchfield & Wilcoxon (1949). Three leaves (replicates) were used for each dose including the checks in each test. Each test was run at least two times and, in some cases, three or four times. The analysis of the dosage mortality data was made on the averages of repeated tests. All calculations were made for significance at the 5% level. The curves of the tests were plotted from the final averages of the repeated tests. Per cent mortalities were corrected by Abbott's formula.

Esterase activity was measured with homogenates of eggs of six different ages. Eggs were laid by mites on bean seedlings held at a constant temperature of 86° F. and held at this temperature until they reached the desired age. The incubation period at 86° F. was from 78 to 84 hours. For the younger egg stages, mites were permitted to lay eggs on bean leaves for periods of 4 hours before the mites were removed. For older egg stages mites were permitted to lay eggs for 12 hours before being removed. Eggs on leaf pieces were held in deep freeze (−23° F.) until enough were collected to be tested. Each replicate comprised 1250 eggs. The eggs were detached from the leaf pieces under distilled water at 41° F., placed in vials, and the excess water removed. The eggs were then homogenized in Clark and Lubs buffer at pH 8.0 using an all-glass grinder at 41° F. The homogenates were filtered through paper using Celite Analytical Filter Aid.

Esterase activity was measured by the method of Archer & Zweig (1959) which uses indophenyl acetate as a substrate. This method requires fewer eggs than the delta pH method using acetycholine as a substrate but is not as specific for cholinesterase activity. The final concentration of indophenyl acetate in the homogenate was 9.6×10^{-5} M. The reaction was allowed to proceed for 90 minutes at 25° C. after which the concentration of indophenol ion was measured with a spectrophotometer. Four determinations were made for eggs of each age group.

RESULTS AND DISCUSSION.—Table 1 shows the survival rates of motile stages of mixed ages during the selection

Table 1.—Per cent survival of mixed motile stages of the two-spotted spider mite after selection pressure treatment of several generations with parathion during the period January 1957 to April 1958.

Parathion Dosage (p.p.m.)	Interval Between Treatments (days)	Total Mites Tested	Survival (%)	Approximate Number of Generations
400	—	2,268	2.2	—
800	37	2,230	3.0	2
	30	1,170	5.1	4
	16	655	10.0	—
	19	472	14.9	9
1,200	19	631	2.5	11
	19	900	5.8	—
	24	511	10.2	15
	15	966	14.2	16
1,600	15	780	4.5	—
	24	1,160	4.9	19
	23	917	6.2	—
	16	1,042	10.1	23
1,800	15	1,235	8.1	—
	27	1,080	9.3	—
	38	1,390	11.3	—
	24	940	11.9	29
2,000	32	1,079	9.3	—
	22	344	11.1	—
	19	1,040	12.0	—
	19	939	13.2	35

period. It is apparent that the per cent survival increased continuously when the mites were dipped in the same concentration of parathion emulsion. When the concentration of parathion was increased the survival rate dropped to a lower level. Table 2 shows the magnitude of resistance to parathion in the adult females and in the young mites. Resistance to parathion was higher in the adult stage than in the young stages. When the selection pressure was relaxed for 8 months, resistance in the females dropped slightly, but when the selection pressure with parathion was stopped for 10 months, resistance in the females dropped to about half the initial magnitude. This result is in agreement with March (1960) who characterizes resistance to organophosphorus insecticides as relatively unstable However, Smith (1960) reported a colony of two-spotted spider mites that had retained their resistance to parathion after 11 years with no selection pressure. Selective pressure applied to any stage of the life cycle of the mites induces resistance in that particular stage and in all other stages. Exposing eggs, larvae, or adults to parathion resulted in resistance to parathion in all of these stages. When only eggs and young mites were exposed to parathion, resistance was detected in the adult females derived from them. The degree of resistance in the young and egg stages, however, was not as great as that of the adults, an observation previously made by Smith & Fulton (1951).

The results of the treatments of the eggs laid by the parathion-resistant and nonresistant populations are shown in table 3. It is clear that the eggs from the parathion-resistant mites are also resistant to parathion. Eggs of the nonresistant mites at several different stages of development were found to be as susceptible to parathion as

Table 2.—Net mortality of parathion-resistant and nonresistant adult females and young two-spotted spider mites when transferred to bean leaves previously dipped in parathion emulsion and dried.

Population Tested	Date of Treatment	Total Mites Tested	Parathion Concentrations (p.p.m.) LD_{50}	LD_{95}	Slope	Degree of Resistance LD_{50}	LD_{95}
Adult mites							
Resistant	7/26/58	396	2,800	9,900	3.01	400	471
Nonresistant	8/21/58	657	7	21	3.31	—	—
Resistant	2/27/59	440	3,150	11,000	3.01	384	407
Nonresistant	1/ 5/59	271	8.2	27	3.31	—	—
Resistant	11/27/59	425	2,010	5,600	2.65	191	160
Nonresistant	11/27/59	370	10.5	35	3.31	—	—
Resistant[a]	7/14/59	453	150	620	2.59	—	—
Young mites							
Resistant	3/ 2/59	410	1,550	5,800	2.91	155	175
Nonresistant	3/ 3/59	713	10	33	3.31	—	—

[a] Resistance developed in adults by exposure of eggs and young stages only.

the adults of this strain. Eggs from parathion-resistant females were resistant to parathion in all stages of development. The eggs of the same strain did not show any significant differences in their response to parathion when treated at different stages of development.

The majority of the log-dosage-probit lines (ld-p lines) of the resistant females, young, and eggs of the mite were steeper than the corresponding lines of the nonresistant females, young, and eggs. These steeper lines may indicate that the resistant population was becoming more homogeneous (Hoskins & Gordon 1956).

Although the eggs of the parathion-resistant mites were definitely resistant to parathion, the females were more resistant than the eggs. The determinations of resistance in the eggs were carried out during the period between the first and second determinations of resistance in the adults, a period in which there was little loss in resistance. Therefore, the higher degree of resistance in the adults as compared with that of the resistant eggs was not due to differences in timing of applications of parathi-

Table 3.—Net mortality of parathion-resistant and nonresistant eggs of two-spotted spider mites treated with parathion at different ages.

Population	Total Eggs Treated	Fraction of Incubation Period When Treated	Parathion Concentration (p.p.m.) LD_{50}	LD_{95}	Slope	Degree of Resistance LD_{50}	LD_{95}
Resistant	475	0–1/3	2,100	9,200	2.54	300	204
Nonresistant	469	0–1/3	7.0	45	2.04	—	—
Resistant	437	1/3–2/3	2,500	18,000	1.95	278	375
Nonresistant	320	1/3–2/3	9.0	48	2.36	—	—
Resistant	385	2/3–1	2,050	15,000	2.14	186	250
Nonresistant	581	2/3–1	11	60	2.23	—	—
Resistant	657	0–1/9	2,250	12,000	2.15	250	261
Nonresistant	342	0–1/9	9	46	2.23	—	—
Resistant	475	1/9–2/9	2,350	8,000	5.22	293	177
Nonresistant	309	1/9–2/9	8	45	2.31	—	—
Resistant	480	2/9–1/3	2,400	13,200	2.32	300	293
Nonresistant	408	2/9–1/3	8	45	2.23	—	—
Resistant	196	2/3–7/9	2,000	8,000	2.62	222	70
Nonresistant	370	2/3–7/9	9	115	1.48	—	—
Resistant	284	7/9–8/9	2,500	11,500	2.54	338	128
Nonresistant	442	7/9–8/9	7.4	90	1.52	—	—
Resistant	230	8/9–1	2,150	17,000	1.90	239	131
Nonresistant	395	8/9–1	9	130	1.50	—	—

Table 4.—Net mortality of parathion-resistant and nonresistant eggs of the two-spotted spider mite treated with several acaricides.[a]

Population	Acaricide	Total Eggs Treated	Acaricide Concentration (p.p.m.)		Slope	Degree of Resistance	
			LD_{50}	LD_{95}		LD_{50}	LD_{95}
Resistant	Parathion	437	2,500	18,000	1.95	278	375
Nonresistant	Parathion	320	9	48	2.36	—	—
Resistant	Malathion	534	1,050	5,000	2.45	2.5	2.4
Nonresistant	Malathion	855	425	2,100	2.35	—	—
Resistant	Diazinon	1,080	52	350	1.98	5.0	6.7
Nonresistant	Diazinon	800	10.5	52	2.36	—	—
Resistant	Tedion	1,074	42	120	3.73	5.8	4.0
Nonresistant	Tedion	556	7.2	29.5	3.11	—	—
Resistant	Kelthane	1,174	65	410	2.04	5.3	4.1
Nonresistant	Kelthane	1,490	12.2	100	1.80	—	—

[a] Eggs treated at ⅓ to ⅔ of incubation period.

on. Also there was no significant difference between the susceptibility of the nonresistant adults and that of their eggs. It seems that the factors involved in mortality caused by parathion interact differently between adults and eggs of the resistant strain as compared with adults and eggs of the nonresistant strain. These factors may include amount of deposit on the eggs and rate of penetration of parathion into the eggs and adults. However, the trend of the present day research on mechanisms of resistance to phosphates puts the emphasis on the biochemical and physiological factors for resistance and considers penetration, lipoid storage, and membrane barriers as secondary factors (March 1959).

The responses of resistant and normal eggs to other acaricides are shown in table 4. In all tests the eggs were from ⅓ to ⅔ developed. The ld-p lines of resistant and normal eggs were parallel for each acaricide tested though the slopes of the lines varied between acaricides. Malathion was ineffective as an ovicide and there was little difference in response between resistant and normal eggs. Diazinon, Tedion, and Kelthane were more effective ovicides but, with these chemicals, the normal eggs were only 4 to 6.7 times as susceptible as the parathion-resistant eggs. In contrast, eggs from resistant females were 278 times as resistant to parathion as eggs of the nonresistant strain at the LD_{50} level.

Table 5.—Mean esterase activity of mite-egg homogenates for indophenyl acetate.

Age of Eggs in Hours	Micromoles Indophenyl Ion Produced[a]	
	Parathion-Resistant Eggs	Nonresistant Eggs
0– 2	14.0 a	7.8 a
0– 4	23.5 b	10.3 ab
8–12	30.7 c	21.8 abc
24–36	34.0 d	29.5 bc
48–60	40.3 e	33.3 c
72–84	57.7 f	56.3 d

[a] Letters indicate significant differences (5%) according to Duncan's Multiple Range Test (1955).

Determinations of egg esterase activity on indophenyl acetate are given in table 5 and figure 1. These results show that esterase activity appeared early in the development of the eggs and increased as the eggs grew older. A statistical analysis of the esterase activity of the eggs of both strains of mites showed the average activity of all six age groups together was significantly higher for the parathion-resistant eggs. The esterase activity for the resistant eggs was also significantly higher in the age groups 0 to 2 hours, 0 to 4 hours, and 8 to 12 hours as compared with corresponding age groups of nonresistant eggs. There were no significant differences in activity between resistant and nonresistant eggs in the more advanced stages of development. Significant differences of eggs of different ages within each strain are indicated in table 5.

The rise in esterase activity in the older eggs may have been due to the increase in cholinesterase accompanying the development of the nervous system. This explanation would agree with the work of Potter *et al.* (1957) who found that in eggs of *Pieris brassicae* L. hydrolysis of acetylcholine was only correlated with the development of the nervous system to a stage where it may become func-

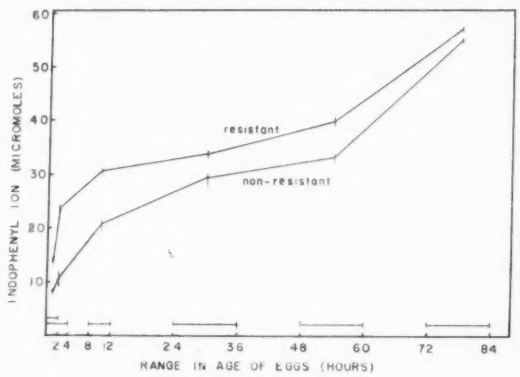

FIG. 1.—Micromoles of indophenyl ion produced from indophenyl acetate in 90 minutes by homogenates of eggs of different ages.

tional. The same authors found that eggs of this moth would hydrolyze phenylacetate during all stages of incubation. Perhaps a similar situation occurs in eggs of the two-spotted spider mite and accounts for the hydrolysis of indophenyl acetate by homogenates of very young eggs.

March (1960) states that there is evidence that organophosphorus resistance in the house fly is not primarily related to higher levels of cholinesterase activity. Van Aspern & Oppenoorth (1959) found less aliesterase in house flies resistant to parathion. The greater esterase activity on indophenyl acetate by parathion-resistant mite eggs is perhaps in disagreement with the work of March and Van Aspern and Oppenoorth on parathion-resistant house flies. However, direct comparisons must await identification of the esterases present in parathion-resistant mite eggs.

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Systemic Insecticides for Control of Aphids on Field-Grown Easter Lily[1]

CHARLES F. DOUCETTE, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

ABSTRACT

At Harbor, Oregon, season-long protection of field-grown lily plants from aphid attack has been obtained from applications over bulbs in the furrow of granulated phorate and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate). Mixing granules in the soil before setting the bulbs was also effective, as well as applying carbon dust formulations directly to the bulbs. Soaking bulbs in dilute emulsions of demeton, phorate, or Di-Syston was effective to a lesser degree. Chiefly involved were the foxgrove aphid (*Myzus solani* (Kltb.)), the melon aphid (*Aphis gossypii* Glov.), and the western lily aphid (*Macrosiphum scoliopi* Essig). The shallot aphid (*Myzus ascalonicus* Doncaster), the potato aphid (*Macrosiphum euphorbiae* (Thomas)), and the green peach aphid (*Myzus persicae* (Sulz.)) appeared in small numbers.

Aphids have been a general and serious problem to growers of Easter lily bulbs. Direct feeding injury is extensive in the absence of control measures, and the aphids also serve as vectors of virus diseases. The use of systemic insecticides for control of aphids on Easter lily plants has been reported by Gill *et al.* (1955). Their objective was to reduce the spread of "fleck," an aphid-transmitted virus complex, and they did not indicate the specific control on the aphids. Since effective systemic bulb treatments would eliminate the expense of aphicidal sprays or dusts, treatments with these materials were started in the fall of 1956 at the Research and Development Station of the Pacific Bulb Growers' Association at Harbor, Oregon.

MATERIALS AND METHODS.—Demeton, schradan, phorate, Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) and Am. Cyanamid 12008 (*O,O*-diethyl *S*-(isopropylthio)methyl phosphorodithioate) were used in emulsions for bulb soaks. Bulbs were dusted with phorate and Di-Syston in activated carbon, and granulated formulations of these two insecticides were applied in the furrows and as side-dressings. The treatments were made in early October, the usual planting time, except for the side-dressings, which were applied in mid-April. The experiments were set up in randomized blocks of four replicates. Five bulbs were planted in each replicate in 1956 and 1957 and six bulbs in 1958. With the exception of the direct bulb treatments (soaking and dusting in paper sacks), the rate of application is expressed in tables 1 to 3 as pounds of toxicant per 12,000 row-feet, which represents the number of row-feet per acre when rows are spaced 42 inches apart. Spacings commonly used in Easter lily bulb production are 36, 40, and 42 inches.

During the growing seasons the aphids on each plant were counted at intervals. The effectiveness of the different treatments was based on a comparison of populations on treated and untreated plants.

In the area where these experiments were conducted

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Table 1.—Number of aphids on 20 Easter lily plants in 1957 following treatment with systemic insecticides the previous fall.

TREATMENT	MAY 21	JUNE 9	JUNE 22	JULY 4	JULY 20	AUG. 4	SEPT. 3
Bulbs soaked in emulsion (lb./100 gal.)							
Demeton, 0.5	38	38	15	48	99	50	[a]
Schradan, 1	73	20	44	61	353	74	[a]
Phorate, 1	41	2	52	44	102	388	160
Am. Cyanamid 12008, 1	62	86	78	84	158	39	115
Water only (check)	27	121	32	55	235	509	900
Phorate granules (3 lb./A.)							
Below bulbs	74	18	74	65	255	154	460
Above bulbs	71	51	53	64	438	599	[a]
Side-dressing[b]	54	55	36	64	116	167	[a]
Untreated (check)	73	66	41	50	290	63	[a]

[a] Most of the plants had matured, and tops were nearly dry.
[b] Applied in April 1957.

approximately 75% of the average annual precipitation of 70 inches falls in the winter and spring. Summers are very dry. Temperatures are mild, rarely below freezing in the winter and maxima averaging 68° F. in the summer, exceeding 80° only a few times. The lily plants emerge in January or February; growth is slow until April, when warmer temperatures induce more rapid development. Aphids are usually not seen on the plants until well along in April.

1956–57 TESTS.—At planting time in the fall of 1956 lily bulbs were soaked for 30 minutes in emulsions containing one-half pound of demeton or 1 pound of phorate, schradan, or Am. Cyanamid 12008 per 100 gallons. The bulbs were still damp when planted. A 2% granulated formulation of phorate at 3 pounds per 12,000 row-feet was mixed in the soil in the furrow bottom before bulbs were placed, or was applied as a band at a level just above the tops of the bulbs, or as a side-dressing in early April.

Counts of aphids were made seven times, starting May 21, and are summarized in table 1. Populations were not high on any of the plots until July 20; at that time only plants treated with demeton, phorate, and Am. Cyanamid 12008 emulsions and phorate side-dressings had few aphids. The August 4 count was irregular largely because of predators. Early maturity of some plants reduced the populations by September 3. Generally the results were inconclusive.

In another experimental series the same season, not intended for aphid control study, a bulb dusting with phorate was included (Jensen *et al.* 1958). The bulbs were shaken in a sack with a dust formulation consisting of 44% phorate in activated carbon. Aphid populations on these plants were very low throughout the growing season.

1957–58 TESTS.—Since the bulb soak treatments of the previous season had not been very effective, stronger emulsions and other methods of application were tested. The soak treatments contained 1 or 4 pounds of demeton and 2 or 8 pounds of phorate per 100 gallons. Dustings consisted of shaking the bulbs in a sack with the following phorate formulations: 44% in activated carbon; 22% or 11% (talc added to dilute); or 22% with 35% PCNB (pentachloronitrobenzene). The actual amount of the dusts that adhered to the bulbs was not determined. Also 10 grams of the 44% phorate dust was applied directly to 20 bulbs after they had been set in the furrow. Applications of 2% granulated phorate were made at the rate of 4 pounds of toxicant to 12,000 row-feet, either mixed in the soil under the bulbs, or placed in a band over partially covered bulbs, or used as a side-dressing in the spring (mid-April).

Aphid counts were made during the growing season and are summarized in table 2. Populations were so decimated by syrphid attack in early August that no counts were attempted after July 16.

On untreated bulbs, the aphid populations started to build up in mid-May, and increased rapidly during June and July. The demeton soak treatments gave considerable protection from aphids, but the mid-July observations indicated that the weaker emulsion was losing some of its effectiveness. Only the stronger emulsion of phorate provided control. All of the bulb dust treatments were highly effective through July. The two fall treatments with granules gave only partial control through June, but the side-dressing in the spring was effective through July. It was later learned that this lot of granules had probably deteriorated before use, so the results cannot be associated with a specific dosage.

1958–59 TESTS.—In the fall of 1958 soak treatments of

Table 2.—Number of aphids on 20 Easter lily plants in 1958 following treatment with systemic insecticides the previous fall.

TREATMENT	APRIL 15	MAY 21	JUNE 29	JULY 16
Bulbs soaked in emulsion (pounds per 100 gallons)				
Demeton, 4	0	9	20	50
1	0	48	40	268
Phorate, 8	0	25	55	172
2	0	44	251	1,486
Bulbs dusted in a sack				
Phorate, 44%	0	16	0	13
22%	0	1	0	2
11%	0	8	2	11
Phorate, 22%+PCNB, 35%	0	7	0	14
Dust on bulbs after setting				
Phorate, 44%[a]	0	10	0	12
Phorate granules (4 pounds per acre)				
Below bulbs	3	80	71	1,062
Above bulbs	0	54	27	723
Side-dressing[b]	0	89	25	92
Check	0	50	347	1,341

[a] 10 grams to 20 bulbs.
[b] Applied in April 1958.

4 pounds of demeton and 8 pounds of phorate or Di-Syston per 100 gallons were evaluated along with different granulated formulations of phorate and Di-Syston and bulb dusts applied in the furrow. The granulated treatments consisted of 8% phorate or 5% Di-Syston applied at rates of 4 and 8 pounds of toxicant per 12,000 row-feet. Granules were either mixed lightly with the soil in the bottom of the furrow before bulbs were set, or were spread along the furrow over the bulbs before they were covered. This latter method differed from the band applications made the previous two seasons since more of the granules were closer to the bulbs. Granules were also applied in mid-April 1959 as a side-dressing. The dust treatments consisted of shaking the bulbs in a sack with 22% phorate or 25% Di-Syston, or applying the dusts to the bulbs in the furrow at the rate of 4 pounds per acre.

Aphids were counted six times between May 12 and August 26, and these counts are summarized in table 3. Late in July an influx of syrphids caused a marked drop in aphid numbers. Excellent protection throughout the season was apparent in all of the Di-Syston treatments except the spring side-dressing. Phorate applied as granules or as a dust to the bulbs produced a very good control. The 8-pound rate of phorate granules and both rates of Di-Syston granules were more effective than the 4-pound rate of phorate. On phorate-treated plants most of the aphids were on the lowest leaves; the colonies did not increase as rapidly as those on the untreated controls. In both the soak treatment and dust application in the furrow, the effectiveness of the phorate began to diminish in July.

Mixing the granules in the soil in the furrow bottom before setting the bulbs appears to be as effective as application over the bulbs. Since the general practice is to set the bulbs by hand this method involves a degree of hazard to the planters. Their hands could come in contact with the insecticide. Possible exposure to the toxic insecticides used as emulsions is involved both in treating and planting.

Table 3.—Number of aphids on 24 Easter lily plants in 1959 following treatments with systemic insecticides the previous fall.

TREATMENT	MAY 12	JUNE 3	JUNE 19	JULY 14	AUG. 5	AUG. 26
Soaked in emulsion (pounds per 100 gallons)						
Demeton, 4	4	7	1	8	56	8
Di-Syston, 8	2	8	0	0	0	0
Phorate, 8	7	57	70	428	203	18
Granules (pounds per acre)						
Di-Syston						
Under bulbs, 4	2	1	5	3	0	0
8	0	0	0	0	3	0
Over bulbs, 4	0	0	0	0	9	0
8	2	2	1	0	0	0
Side-dressing, 8[a]	52	177	56	306	192	122
Phorate						
Under bulbs, 4	18	32	132	30	92	7
8	5	2	0	0	39	2
Over bulbs, 4	13	10	4	71	19	2
8	3	0	1	3	11	0
Side-dressing, 8[a]	53	119	127	1,081	326	239
Bulbs dusted						
In furrow (4 pounds per acre)						
Di-Syston	0	2	8	0	0	0
Phorate	3	88	70	631	166	0
In a sack						
Di-Syston, 22%	1	0	0	0	12	0
Phorate, 22%	4	12	29	12	11	0
Check	66	152	590	5,916	1,131	383

[a] Applied in April 1959.

Table 4.—Numbers of aphids of various species found on Easter lily plants.[a]

DATE	*Macrosiphum scoliopi*	*Myzus solani*	*Aphis gossypii*	*Myzus persicae*	*Myzus ascalonicus*
1957					
May 21	0	575	10	—	0
June 9	0	457	0	—	0
22	19	442	0	—	0
July 4	104	364	91	—	0
20	1,703	156	343	—	44
Aug. 4	2,074	10	7	—	14
1958					
May 21	0	440	1	—	0
June 29	694	49	88	—	0
July 16	5,924	172	132	—	2
1959					
May 12	8	134	33	59	1
June 3	111	349	214	3	0
19	590	220	284	10	0
July 14	8,278	5	166	29	0
Aug. 5	2,210	0	60	0	0
26	536	2	153	0	0

[a] 9 *Macrosiphum euphorbiae* in 1957 and 1959.

The changes in the make-up of the aphid populations during the three summer seasons are shown in table 4. The predominant species encountered were the western lily aphid (*Macrosiphum scoliopi* Essig), the foxglove aphid (*Myzus solani* (Klth.)), and the melon aphid (*Aphis gossypii* Glov.).

The western lily aphid appeared in the cultivated plants in late spring and preferred the under sides of the lowest leaves. This species, with its high reproductive capacity, densely populated the plants by mid-July. Heavy feeding by the insects caused the lower leaves to become dry; at this point the aphids moved upward on the plant. This species can damage a plant so seriously that it eventually dies.

The foxglove aphid preferred young leaves and buds in the developing tips. It moved into the lily plants in April and was the only species present for some time; but when the buds began to spread, it transferred to other hosts.

The melon aphid preferred the upper leaves and was usually found in irregular formation on the edges of the under sides in May, developed slowly throughout the summer, and reached its peak in September, about harvesttime. It seemed to be susceptible to predators.

Other species found were the shallot aphid (*Myzus ascalonicus* Doncaster), the potato aphid (*Macrosiphum euphorbiae* (Thomas)), and the green peach aphid (*Myzus persicae* (Sulz.)). They occurred only as small colonies and were scattered on the plants.

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SCIENTIFIC NOTES

Melon Aphid Control on Cantaloupes[1]

JAMES A. HARDING[2]

The melon aphid, *Aphis gossypii* Glover, commonly infests watermelons, cantaloupes, and cucumbers which are all major crops in South Texas. Damage by this insect is often spotty over the area ranging from endemic to epidemic in proportion.

Parathion is commonly used for aphid control. A test was conducted on September 20, 1960 to compare the effectiveness of some of the newer insecticides with parathion when applied in low gallonage sprays as is commonly practiced locally. Reports on chemical control studies against the melon aphid on vine crops are few in recent economic literature; however, there is extensive information concerning the control of this insect on cotton.

Recent studies by Michelbacher *et al.* (1955) indicated that an application of 0.6 lb. of parathion or 1.1 lb. of malathion per acre resulted in good control of this insect and that dieldrin, aldrin, and heptachlor showed similar promise. Ditman *et al.* (1957) found that the use of 4% malathion, 2% Dilan, or 2% lindane aerosols were effective for use by the home gardener. Young & Ditman (1959) obtained effective control of the melon aphid on cantaloupes with 0.44 lb. Sevin®, 0.28 lb. Thiodan®, 0.56 lb. malathion, and 0.26 lb. Trithion® per acre in an average of 27 gal. of spray per acre. Poorer control was obtained with 0.28 lb. ethion and 0.14 lb. Dibrom® per acre. No yield increases were associated with this control. Hills & Taylor (1959) recommended 0.6 lb. of parathion or demeton per acre for control of the melon aphid on cantaloupes.

MATERIALS AND METHODS.—At the time of application, the plants had vines averaging 15 inches in length and blooms were present. Some curling of the leaves was evident with an average of 98 aphids per leaf. All sprays were applied when there was no appreciable wind movement. Furrow irrigation was used with rows located on beds having 6-foot centers.

The design of this test was a randomized block having each treatment replicated three times on experimental plots 1 row wide by 40 to 45 feet long. The treatments were evaluated by recording the number of live aphids on 10 randomly selected leaves per plot. No check plots were included in this test due to the high crop value of this planting.

Records were made 1, 3, 7, 10, and 16 days after treatment. Data were analyzed for sources of variance by applying the F test and differences between the treatment means were analyzed by using Duncan's New Multiple-Range Test.

Each insecticide was applied in spray form at the rate shown in table 1. Two 70-degree flat-fan nozzles per row directed to hit the undersides of the leaves and calibrated to deliver 10 gallons of formulation per acre at a pressure of 30 p.s.i. were used.

The insecticides included in this test were parathion, dimethoate, ronnel, ethion, phosphamidon, demeton, phorate, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thiophosphate), Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Trithion® (*S*-[*p*-chlorophenylthio) methyl] *O,O*-diethyl phosphorodithioate), Methyl Trithion® (*O,O*-dimethyl *S*-[(*p*-chlorophenylthio) methyl] phosphorodithioate), and Dylox® (dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate).

RESULTS AND DISCUSSION.—Results of this test are summarized in table 1. Analysis shown is of these tabular means which indicates the most effective chemical based on the duration of the test. Date versus treatment interaction was highly significant but no further breakdown of the data was deemed necessary.

Demeton, phorate, dimethoate, phosphamidon, ronnel, and Diazinon in the present test were more effective than parathion, which is commonly used for aphid control. Diazinon did not differ from Trithion but was superior to parathion while Trithion equaled parathion. Methyl Trithion, Dylox, ethion, and Dibrom were less effective than parathion; however, numerically it appears that Methyl Trithion gave control equal to parathion for 7 days, but became ineffective 3 days later.

Table 1.—Effectiveness of several insecticides against the melon aphid on cantaloupes. 1960.

Toxicant	Toxicant per Acre (lb.)	Mean No. of Aphids per 10 Leaves[a] on Days After Treatment Shown 1	3	7	10	16	Results of Analysis[b]
Demeton	0.25	2	3	1	0	51	
Phorate	.25	9	6	0	2	46	
Dimethoate	.25	5	1	1	7	84	
Phosphamidon	.25	2	3	16	8	154	
Ronnel	.50	2	1	1	3	189	
Diazinon	.75	6	1	16	21	191	
Trithion	.50	54	158	36	95	229	
Parathion	.25	27	54	54	103	400	
Methyl Trithion	.50	18	81	37	538	603	
Dylox	.75	76	359	185	946	860	
Ethion	.50	49	821	1,105	728	—	
Dibrom	.50	97	917	1,127	1,591	—	

[a] To the nearest whole number.

[b] Over-all analysis of these means equalling significant differences if not paralleled by same line.

Table 2.—Plant area of effectiveness against the melon aphid on cantaloupes. 1960.

Toxicant and Date of Record		Mean No. of Aphids per 10 Leaves[a] Old Foliage	New Foliage
Demeton	10/6	26	76
Phorate	10/6	66	26
Dimethoate	10/6	97	72
Phosphamidon	10/6	218	90
Phosphamidon	9/27	26	6

[a] To the nearest whole number.

Winged aphids were present until September 30. Partial counts and general observations would suggest a high degree of correlation between the abundance of winged adults and the poorer insecticides.

The convergent lady beetle, *Hippodamia convergens* (Guér.-Mén.), was also abundant at the beginning of the test and after the aphids built up again. Records indicated that no beetles were present in any of the plots until 7 days after treatment. At this time partial counts showed that more lady beetles were present in the plots where aphid control was poor.

Aphid counts on old versus new foliage are shown in table 2. It would appear from these records that phosphamidon, phorate and, to some extent, dimethoate were more effective in reducing aphids on the new foliage than where the spray deposits originally contacted the plant. The effectiveness of demeton remained greatest where the spray hit and was not translocated as readily to the new foliage as was the case with the other toxicants in this test.

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[2] Assistant Entomologist, Texas Agricultural Experiment Station, Crystal City.

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Effects of Mixtures of Cubé with Various Drugs on the Mexican Bean Beetle[1]

F. H. HARRIES, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

Derris, cubé, and other rotenone products were for many years the most effective materials available for control of the Mexican bean beetle (*Epilachna varivestis* Muls.). Such insecticides are notably slow acting, and in this insect the effects are characterized by early cessation of feeding with delayed mortality. Possibly some of this effect may be caused by paralysis of the mouthparts or feeding processes so that death results more directly from desiccation. This is indicated by shriveling and decrease in size of the larvae before death and by the long time often necessary for the affected adults to die. Feeding may also be stopped by sublethal dosages since beetles treated with these dosages often resumed feeding and laid fertile eggs when placed on untreated bean plants. Since feeding paralysis may occur before they consume a lethal dose and because of the slow toxic action of minimal dosages, some common drugs were tested in mixtures with cubé to find if feeding of the insects or effectiveness of the insecticide could be increased. Also, it was supposed that effects of the drugs might show something of the mode of action of derris or rotenone. The tests were made in 1947 in Columbus, Ohio.

The slow toxic action of rotenone was illustrated by mortality data at 5-day intervals after treatment where 15, 34, 48, 53, 49, 42, 15, 25, and 0 were the percentages of control as compared with the untreated checks. A maximum of 53% mortality after 20 days indicated that some kill occurred this late after treatment. These data were based on successive examinations of 10 groups of 10 adults at 80° F. and relative humidity of 50%.

Since the time necessary to effect kill of the adults and also the percentage of recovery seemed to depend considerably on the amount of poison encountered or eaten by the beetles before knockdown occurred, other tests were made in which the plants were treated with different amounts of cubé dusts at strengths of both 0.5% and 1% of rotenone. The results given in table 1 show that mortality generally occurred earlier on plants receiving the stronger dust and on those with the heavier dust deposits.

Rotenone has been found to depress oxygen consumption, respiration, and heartbeat in certain insects and is generally classified as a neuromuscular or muscular depressant with neurotoxicity (Tischler 1935, Hamilton 1939, Lord 1949, Harvey &

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Table 1.—Mortality of adult Mexican bean beetles and area of bean leaf eaten per insect in indicated days after applying different amounts of cubé-pyrophyllite dusts containing 0.5% and 1.0% of rotenone.

Per Cent of Rotenone	Mg. of Dust Applied	Mortality (%) After Indicated Days						Square Centimeters Eaten at Indicated Days					
		3	6	9	12	15	18	3	6	9	12	15	18
0.5	50	4	4	4	14	26	36	0.5	0.6	1.6	2.2	4.0	5.3
	100	0	2	20	40	54	64	0	0	0	0	0	0
	150	6	12	38	52	72	78	0	0	0	0	0	0
	200	16	28	56	56	70	78	0	0	0	0	0	0
	250	10	24	40	64	80	88	0	0	0	0	0	0
1.0	50	14	24	36	44	64	74	0	0	0.1	0.1	0.1	0.1
	100	2	20	36	50	68	76	0	0	0	0	0	0
	150	14	30	48	70	80	82	0	0	0	0	0	0
	200	18	34	54	72	80	88	0	0	0	0	0	0
	250	32	52	80	86	90	88	0	0	0	0	0	0
Check	0	4	4	8	12	24	38	0.8	3.2	4.8	8.8	12.1	16.9

Table 2.—Mortality of Mexican bean beetle larvae and adults and square centimeters of bean leaf area eaten per insect in indicated days after applying dusts containing 0.5% of rotenone with and without 2% of different drugs.

Treatment	Mortality (after days shown) of			Feeding (after days shown) in Square Centimeters of Leaf Area		
	Larvae	Adults		Larvae	Adults	
	4	5	10	4	5	10
Rotenone plus						
Physostigmine salicylate	91	35	70	0.3	0.0	0.2
Benzedrine sulfate	86	42	80	0.4	0.0	0.0
Phenobarbital sodium	86	40	80	0.5	0.0	0.1
Evipal (hexobarbital)	90	35	70	0.3	0.0	0.1
Metrazal (pentylenetetrazol)	93	37	80	0.3	0.0	0.0
Rotenone without drug	96	47	85	0.0	0.0	0.0
Check	0	20	20	3.5	4.9	8.4
L.S.D. at 5%	9	23	15			

Brown 1951, Orser & Brown 1951). Insects with a nervous system similar to the parasympathetic autonomic system of vertebrates have been found to react to stimulant drugs such as pilocarpine and physostigmine (eserine) and sedatives such as Nembutal® (Sodium 5-ethyl-5-(1-methylbutyl)-barbiturate) and phenobarbital (Brown 1951).

Results of tests with cubé combined with different drugs are given in table 2. All of the feeding stopped when cubé alone was applied, but there was slight feeding of both larvae and adults when different drugs were added. Physostigmine (alkaloid from Calabar bean) has been found to stimulate nervous and muscular activity in insects (Brown 1951) and may have increased the amount of feeding slightly, but there was a small decrease in mortality instead of an increase as might be expected from the ingestion of more toxicant. Physostigmine has also been found to have some insecticidal action (Tattersfield 1927). With Benzedrine® (dl-α-Methylphenethylamine), a stimulant to the central nervous system in medical use, a significant decrease in mortality of the larvae occurred compared with the cubé dust without the drug. Mortality of the larvae also decreased significantly with the addition of phenobarbital, which may have a sedative effect on insects (Brown 1951). In the tests of Evipal® (5-(1-Cyclohexen-1-yl)-1-5-dimethylbarbituric acid), a sedative, and Metrazol® (6,7,8,9-tetrahydro-5-H tetrazoleazepine), a stimulant to the nervous system in medical use, there were no marked or significant effects of the drugs. Data for the adults like those for the larvae (table 2) indicate that toxicity of the rotenone dust was slightly reduced by addition of the drugs. Data for the adults after the 10-day observation period indicate that mortality was reduced significantly by physostigmine and Evipal®. Since low dosages of drugs may excite, whereas higher doses may depress activity, a possibility exists that more marked effects might have resulted at other dosage levels.

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Airplane Applications of Malathion Bait Spray for Mexican Fruit Fly Control[1]

J. G. SHAW, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

Malathion in combination with protein hydrolysate bait (Steiner 1952) has been applied in dilute and concentrated sprays to prevent establishment of the Mexican fruit fly (*Anastrepha ludens* (Loew)) in the Tijuana area of Mexico and Southern California since 1955 (Shaw 1955). A partially hydrolyzed yeast protein, the first such material used in this area control program, was somewhat attractive to the fruit flies, but significant differences in the performances of malathion sprays with and without this bait could not be demonstrated in subsequent field tests at four widely distributed areas in Mexico (Shaw & Spishakoff 1958). After Staley's corn protein hydrolysate (SIB No. 7) appeared to be more attractive than partially hydrolyzed yeast in single-tree plot tests (unpublished data), the corn protein material was substituted for the partially hydrolyzed yeast in the Mexican fruit fly formulation. However, convincing evidence of the superiority of a malathion spray containing the new bait over malathion alone was still lacking until the present tests were undertaken in the Montemorelos area of Mexico.

MATERIALS AND METHODS.—Malathion 25% wettable powder at the rate of 2 lb. alone and with 1 quart of SIB No. 7 bait in water to make 2 gallons of spray was applied with a Piper Cub airplane at the rate of 2 gallons per acre. Each treatment was replicated three times; five applications were made to each plot at 10- to 11-day intervals between March 21 and May 3. The plots ranged from 6 to 16 acres in size. Conditions were generally favorable with dry weather prevailing throughout the test period.

Table 1.—Effect of aerial applications of corn protein hydrolysate-malathion bait sprays on Mexican fruit fly infestation in grapefruit.

	TREATMENT[1]		
	Malathion Alone	Malathion Plus 1 Quart SIB No. 7 Corn Protein Hydrolysate	None
Number of grapefruit	49,000	45,415	27,500
Infested grapefruit	1,045	217	2,810
Percentage infested	2.0	0.5	10.2
Percentage reduction in infestation	80.7	95.4	—

[1] Ingredients in water to provide 2 gallons per acre: 25% malathion wettable powder at 2 lb. per acre.

Twice during each interval between sprays invaginated glass traps baited with a fermenting lure containing 8% of light-brown sugar, 0.15% of brewers' yeast, and 0.1% of pyridine in water were used to check adult fly density within a marked central portion of each plot. Infestations in these same areas were calculated from the estimated crop on the trees and the fallen fruit yielding fruit fly larvae.

RESULTS.—Only one-fourth as many flies were caught in plots (eight traps in each) treated with the malathion-bait spray as in those receiving malathion alone. Compared with catches in untreated areas, the reductions were 98.1% and 92.8%, respectively. The 10- to 11-day interspray interval appeared to be satisfactory for effective protection of the crop, but this length of time may have represented the limit of effectiveness of the malathion since catches increased 10 to 15 days after treatments were discontinued. Of females trapped between March 7 and May 8, 64% were gravid; 26% of those trapped between May 8–21 were gravid. This difference in fertility reflected the accumulation of large numbers of newly emerged flies that developed during the favorable host period in April.

The definite superiority of the bait-spray combination was also demonstrated by reductions in fruit infestation of 95.4% in the malathion-SIB No. 7 plots compared with 80.7% in plots receiving only malathion (table 1). Fruit infestation in the untreated plots continued to increase until April 30 but after that date declined, possibly because of drought and hot weather.

Aerial spraying of the malathion-SIB No. 7 formulation was responsible for the successful eradication of the Mediterranean fruit fly in Florida in 1956–57 (Steiner *et al.* 1961). The present

[1] Accepted for publication January 25, 1961.

evidence suggests the same formulation will be equally effective if applied by air to incipient infestations of the Mexican fruit fly.

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Long-Term Effectiveness of Soil Insecticides on the Alfalfa Snout Beetle Larva[1]

H. Y. FORSYTHE, JR., C. S. KOEHLER, and GEORGE G. GYRISCO, *Cornell University, Ithaca, New York*[2]

The alfalfa snout beetle, *Brachyrhinus ligustici* (L.), is an important insect pest of alfalfa in Jefferson and Oswego Counties, New York. The larva spends nearly 2 years in the soil, feeding on the roots and causing severe damage to the plants. It has been shown (Koehler *et al.* 1958) that certain insecticides applied to the soil as dusts and granules gave excellent control of the alfalfa snout beetle. Plant persistence counts also were found to be higher over a period of about 2 years in plots treated with high dosages of aldrin, dieldrin, and heptachlor dusts.

The results reported herein deal with the long-term effectiveness (about 4 years) of low dosages of some granulated insecticides against the alfalfa snout beetle and the persistence of alfalfa plants.

METHODS AND MATERIALS.—On June 11, 1956, aldrin, dieldrin, and heptachlor granules, each at rates of 5.0 and 3.0 pounds per acre of actual toxicant, were applied to an alfalfa seeding with a hand-operated fertilizer spreader. The experiment, consisting of a randomized complete block design with eight blocks and seven treatments, was conducted at St. Lawrence, N. Y. The plots were 1/200 acre in size.

Six 1-square-foot holes were dug to a depth of 18 to 24 inches in each plot on November 7, 1959. Each sample was selected in a systematic manner so as to include at least one alfalfa plant. The soil in each sample was then hand-sorted and the number of larvae counted. In addition, the living plants in each plot were counted. Only seven blocks were sampled because one block contained too few alfalfa plants for sampling.

The data were analyzed with the analysis of variance procedure after first transforming the plot counts of the number of larvae to the square-root scale. The means of the larval counts were then compared with Duncan's multiple range test at the 5% level.

RESULTS.—The results of the counts of alfalfa snout beetle larvae and living plants are presented in table 1. The insecticides that were found to give excellent control after a period of about 4 years were dieldrin at 5.0 and 3.0 pounds per acre, aldrin at 5.0 pounds, and heptachlor at 5.0 pounds. Heptachlor and aldrin, each at 3.0 pounds per acre, gave relatively poor control.

Plant persistence counts show that there were on the average twice as many alfalfa plants in the insecticide-treated plots as there were in the untreated plots. However, it should be pointed out that all plots showed an extremely low number of plants, which may have influenced the larval counts to some degree.

Table 1.—Larval and plant persistence counts made in plots treated with granulated aldrin, dieldrin, and heptachlor. St. Lawrence, Jefferson County, N. Y., 1956–1959.

INSECTICIDE[a]	ACTUAL TOXICANT (LB./A.)	AVERAGE NUMBER OF: Plants per 1/200 Acre	AVERAGE NUMBER OF: Larvae per Sq. Ft.[b]
Dieldrin	5	10.6	0.1
	3	12.0	0.3
Aldrin	5	9.9	0.4
Heptachlor	5	11.4	0.5
	3	10.0	2.1
Aldrin	3	10.3	2.9
Untreated	—	5.4	4.1

[a] Applied June 11, 1956.

[b] Sampled November 7, 1959. Any two means flanked by the same line are not significantly different at the 5% level.

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[2] The authors gratefully acknowledge the assistance of the following men in much of the field work: R. L. Ridgway, E. W. Huddleston, G. O. Poinar, Jr., R. J. Little, Jr., S. W. Jacklin, G. Levesque, R. J. Prokopy.

Modification of a Commercial High-Clearance Sprayer for Use in Small Plot Spraying[1]

L. H. WILKES AND J. K. WALKER, JR.[2]

The development of low gallonage insecticide sprays for the control of cotton insects brought with it the need for a suitable sprayer for researchers. Tractor-sprayers, while satisfactory for large fields, were not suitable for small-plot tests involving the comparison of a number of insecticides. Hand sprayers have been used, but the exposure of the operator to the materials can be excessive. The exposure problem has become of more concern in recent years with the increased testing of organophosphate insecticides. Clearly, an efficient spray device was needed by workers engaged in the field testing of insecticides.

Confronted with the limitations of the hand sprayers and conventional tractor sprayers, entomologists conducting insecticide comparisons at the Texas Agricultural Experiment Station began using some of the commercially available, high-clearance, self-propelled sprayers. In order to employ these machines in plot work, several modifications have been made. These changes, reported herein, are the incorporate ideas gained from entomologists and agricultural engineers working together with the equipment over a period of several years.[3]

The principal change has been in the spraying system of the machine with little or no modifications in the structural framework of the sprayer. Therefore the changes can be adapted to most any commercially available high-clearance sprayer.

The main supply tank is replaced by a rack made of angle iron

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[2] Respectively, Associate Professor, Agricultural Engineering Department, and Assistant Professor, Entomology Department.

[3] The following individuals contributed to the development of the modifications: R. L. Hanna, D. F. Martin, M. G. Davenport, W. T. Magee, N. M. Randolph, and E. C. LaBarre.

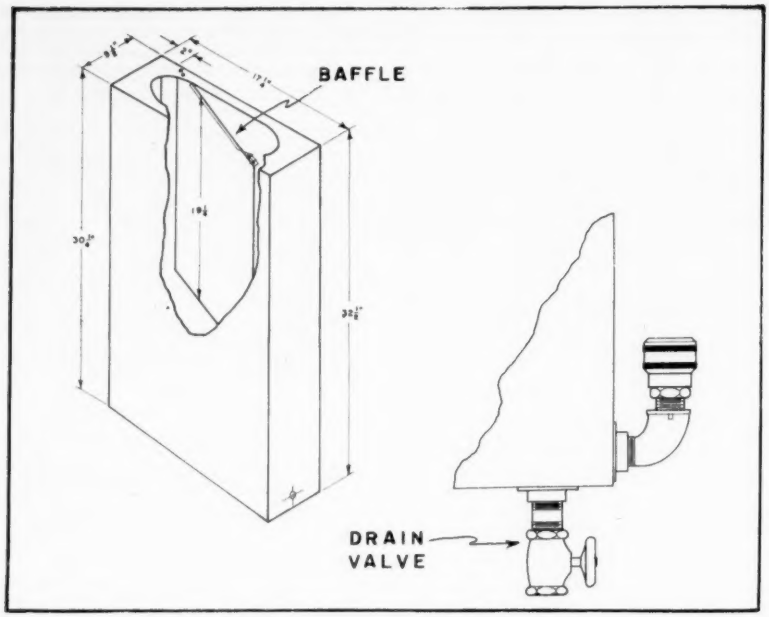

FIG. 1.—Ten-gallon capacity tank, specially constructed for use in small field plots with commercial high-clearance sprayers. Rectangular construction was selected so that maximum utilization of space could be realized.

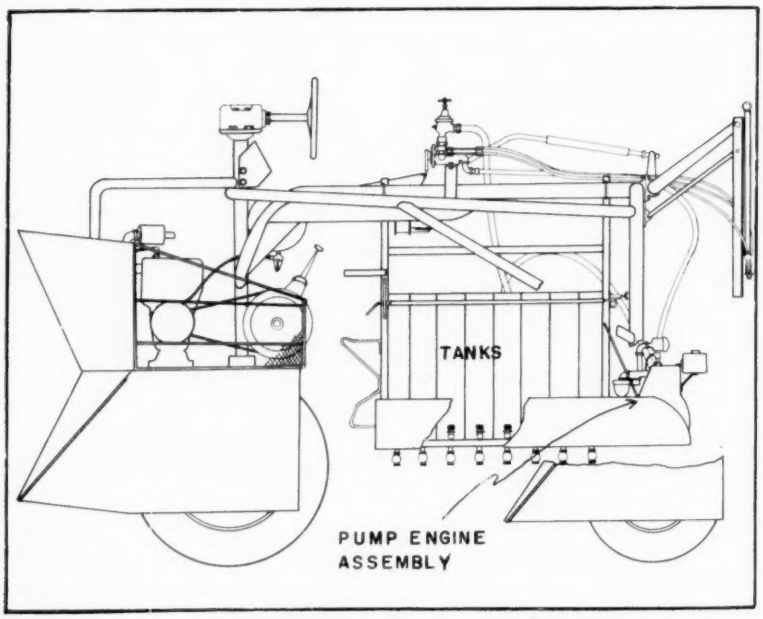

FIG. 2.—Schematic drawing showing the location of the tanks in the tank rack which replaced the original supply tank. The pump engine is mounted on the rear of the tank.

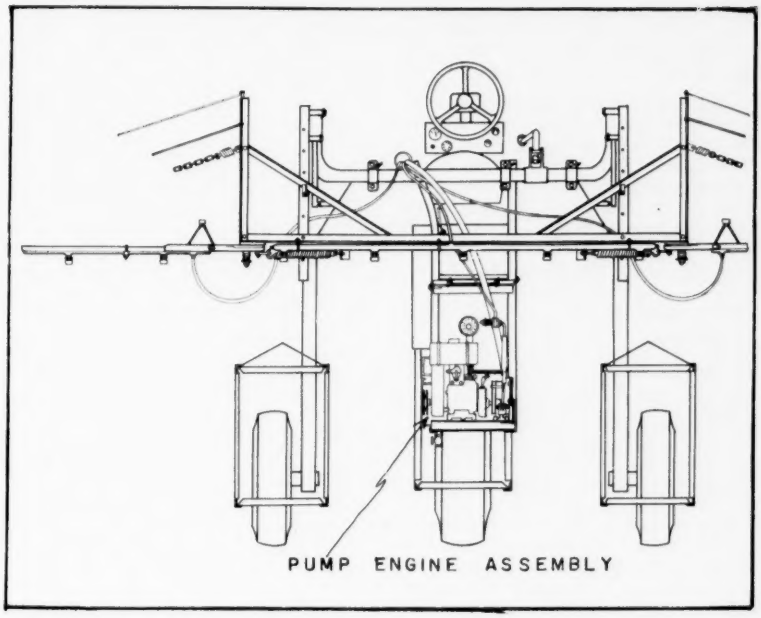

FIG. 3.—Rear view of sprayer showing the location of the pump engine assembly.

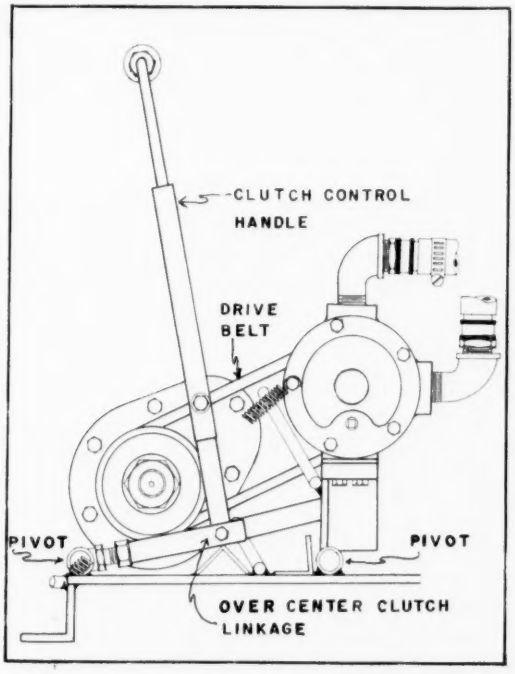

FIG. 4.—A schematic diagram of the principal working parts of the over-center clutch arrangement. To engage the pump, the linkage causes the pump to rotate on the pivot point so that the drive belt is tightened.

which is used to hold several smaller tanks. These tanks, as shown in figure 1, are made of galvanized iron. The rectangular pattern permits as many as eight tanks to be stacked neatly in the rack which has replaced the original supply tank. Tanks having the dimensions as shown in figure 1 will hold up to 10 gallons of material without excessive loss from splashing. A baffle in each tank helps to minimize splashing. The top of each is left open to permit easy and rapid filling. The tank bottoms are angled slightly so that the unused materials can be drained easily through a valve on the lower edge. A second outlet is incorporated in the side near the bottom for the intake hose of the pump. A quick connecting valve is used to permit rapid change from one tank to another.

The second major change was to relocate the pump and auxiliary engine. As may be noted in figures 2 and 3, these components are located on the rear of the tank rack. At this location the pump is at or below the liquid level in the tanks, therefore reducing the chance of pump priming difficulties. At this lower position, the engine is more accessible for servicing and starting.

The pumps are generally connected directly to the auxiliary engine as the sprayer comes from the factory. However, for plot work, it is desirable to have some means of stopping the pump when changing insecticides without stopping the engine. The simple over-center clutch arrangement with a "V" belt drive, figure 4, has been found to work exceptionally well for this purpose. The pump is mounted on a hinged bracket and is actuated by the over-center linkage. To engage the pump, the linkage rotates the pump assembly about the hinged bracket until the drive belt is tightened. The pump is disengaged by reversing the linkage releasing the belt tension. The linkage is adjustable to account for normal belt wear.

The plot width used at the Texas Station is normally 12 rows. The spray boom has been extended so that the 12 rows are sprayed. This was accomplished by adding 2-row extensions at each end of the boom.

A Portable Sprayer for Experimental Plots[1]

RALPH SCHOPP and B. J. LANDIS, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2]

Aphids and spider mites often require control on potatoes in eastern Washington where the continuous use of irrigation water and the tangled plant growth late in the season preclude the use of conventional ground applicatory equipment. In order to test a large number of new pesticides in small replicated plots at low-gallonage rates, comparable to aircraft applications, a portable boom-type sprayer was constructed at the Union Gap, Washington, laboratory. The use of carbon dioxide as a spray propellant for small experimental sprayers was first proposed by Taylor & Hills (1953).

The construction of the sprayer is shown diagrammatically in figure 1. The machine consists of a boom light enough to be carried by two men, and a 1- ×4-inch wooden block panel supporting a carbon dioxide cylinder and spray tank to be carried by a third man. Carbon dioxide, held in the cylinder (*A*) under 800 p.s.i. pressure, is released into the system through a cut-off valve (*B*) to force the spray out of a small tank (*G*). A pressure-regulating valve (*C*) is set as indicated by a gauge (*D*). A valve (*E*) releases the gas into the spray tank, and a safety valve (*F*) prevents excess pressure from building up. When the spray is loaded, valves *E* and *K* are closed and valves *J* and *H* are opened. The spray is then poured into the tank through an attached funnel (*I*), valves *J* and *H* are closed and valve *E* is opened. Valve *K* is then opened to begin spraying. This equipment is mounted on both sides of the panel.

The boom (*L*) is 16 feet long and hinged in the center to allow for folding during transportation. It is of channel-beam construction with its top and bottom of 1- ×2-inch pieces of wood connected by a webbing of $\frac{1}{4}$-inch plywood. The boom tapers from a height of 6 inches in the center to 3 inches at each end. Carrying handles (*M*), of $\frac{5}{16}$-inch rods, fastened to the ends make it pos-

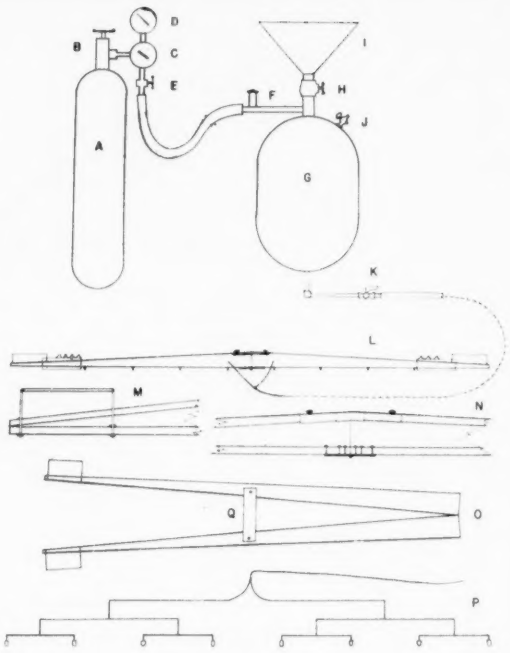

FIG. 1.—Schematic diagram of portable boom-type carbon dioxide sprayer. *A*, carbon dioxide cylinder; *B*, cut-off valve; *C*, pressure-regulating valve; *D*, pressure gauge; *E*, line valve; *F*, safety valve; *G*, spray tank; *H*, spray tank fill valve; *I*, funnel; *J*, relief valve; *K*, boom line valve; *L*, extended boom; *M*, hand hold; *N*, boom-folding mechanism; *O*, folded boom; *P*, spray distribution diagram; *Q*, supporting brace.

[1] Accepted for publication January 25, 1961.

[2] In cooperation with the Washington State Potato Commission and the Washington Agricultural Experiment Station.

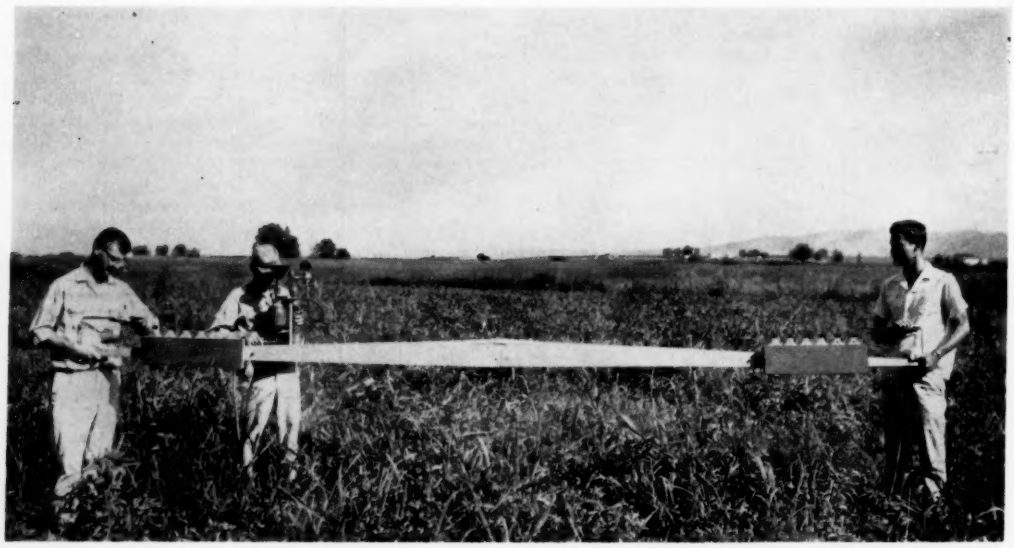

FIG. 2.—Portable boom-type sprayer operating in a potato field.

sible to keep the boom in the proper position while in operation. Wooden boxes attached near the ends of the boom provide space for twelve 1-pint bottles of spray so that the men may treat several plots without new supplies.

The folding mechanism of the boom is shown in an extended position in *N*. The bottom of the boom is held together in the center by a strap hinge. The top is held together and supported in the extended position by a channel-shaped piece of aluminum which fits snugly and is secured by a bolt and wing nut on each side. The same piece of aluminum (*Q*) also serves to hold the boom firm when it is folded, as in *O*.

In order to reduce unequal delivery of spray all nozzles are placed the same distance from the spray tank as shown in *P*. The spray hose from the tank to the boom, the Y-shaped connection, and the copper tubing from it to each side of the boom are $\frac{3}{4}$-inch in diameter; the secondary branches are $\frac{5}{16}$-inch and the copper tubing to the nozzles is $\frac{1}{4}$-inch.

There is no place for spray to collect in the system; when the spray is changed, traces of the previous spray are flushed out at the beginning of the plot. This end of the plot can be avoided in sampling.

The boom is designed to spray four rows of potatoes spaced 34 inches apart. Two nozzles are provided for each row and the nozzles are spaced at 17-inch intervals. With Monarch H-584 No. 3 nozzles at 50 p.s.i., one-half pint of very fine spray can be applied in 10 seconds. A stopwatch is used to regulate the speed of travel, determined by this rate of application. The equipment is shown in operation in a potato field in figure 2.

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Endrin Residues in the Fat of Lambs Grazed on Endrin-Treated Pasture[1]

W. H. LONG, L. D. NEWSOM, and A. M. MULLINS,
Louisiana State University, Baton Rouge

Endrin was recommended in 1958 for sugarcane borer (*Diatraea saccharalis* (F.)) control, and has been widely used since then. Four broadcast applications per acre of 12 pounds each of 2% endrin granules are commonly made at 2-week intervals during the growing season.

A few sugarcane growers who were accustomed to grazing sheep in their cane fields for grass control have also desired to use endrin for borer control. To determine what effects the use of endrin might have on these animals and how much endrin might be found in the fat of such animals at different intervals after access to treated areas, 12 young lambs in poor condition were obtained. The test was conducted in grass pastures instead of sugarcane fields because of convenience and also to increase the chances of harming the sheep and contaminating their meat.

Six lambs were placed on each of two grass pastures May 19, 1958. Each pasture was approximately one-half acre in size and was mowed several times during this experiment in an attempt to maintain the height of the grass at approximately 6 inches. Shade was provided by trees at one end of the pastures. A salt box and watering trough were placed in each pasture, but no additional feed was provided.

Two per cent endrin granules were applied broadcast to one of the pastures on the following dates: May 19, June 1, June 10, June 16, June 23, and June 29, 1958. One-fourth pound of actual endrin was applied to the treated pasture at each application. A Cyclone seeder was used to apply the insecticide granules during the early morning while the grass was wet with dew.

The lambs were observed daily for signs of sickness, but no symptoms were noticed. On July 14th after 55 days of continuous grazing on the pastures the two largest lambs in each group were butchered to obtain fat samples for endrin residue analyses. The remaining animals in the treated pasture were transferred to the untreated pasture at this time. Ear tags were used to distinguish those which had grazed on the treated pasture from those which had grazed continuously on the untreated pasture. Half of the remaining lambs were butchered July 28 and the remainder August 25, 1958, or 14 and 42 days, respectively, after removal from the treated pasture.

Table 1.—Weights of lambs before and after grazing on endrin treated and untreated pastures at Baton Rouge, Louisiana, 1958.

Total Number of Days on Pasture	Live Weight (lb.)				Increases in Pounds per Lamb per Day	
	Treated		Untreated			
	Initial	Final	Initial	Final	Treated	Untreated
55	35	53	40	52	0.33	0.22
	45	60	47	63	0.27	0.29
69[a]	51	62	46	58	0.16	0.17
	47	64	46	62	0.25	0.23
97[b]	58	68	72	80	0.10	0.08
	58	68	54	67	0.10	0.13
Means	49	63	51	64	0.20	0.19

[a] Lambs from treated group grazed on treated pasture 55 days followed by 14 days on untreated pasture.

[b] Lambs from treated group grazed on treated pasture 55 days followed by 42 days on untreated pasture.

Table 2.—Endrin residues found in lamb fat from animals allowed to graze for 55 days on endrin-treated pasture, Baton Rouge, Louisiana 1958.

Days of Feeding on Untreated Pasture	Endrin in Fat (p.p.m.)	
	Internal	External
0	18.3	11.5
	23.4	14.0
14	23.7	20.1
	20.3	14.6
42	8.9	6.4
	13.8	11.0

Each lamb was weighed initially and again at the time of slaughter. The data in table 1 show that all of the lambs gained weight regardless of whether they grazed in the endrin-treated pasture. There was no significant difference in average weight increase between the two groups.

Samples of "internal fat" were taken from around the stomach and from the thoracic cavity of each lamb at the time of slaughter. "External fat" samples were taken from the external surfaces of the carcasses and from fat pockets in the meat after the carcasses had aged for 4 days at 36° to 38° F. All fat samples were frozen and shipped for analyses to the Shell Development Company laboratories at Modesto, California.

Amounts of endrin present in the fat were determined by the chromatographic dechlorination phenyl azide method described by Bann *et al.* (1958).[2]

Results of analyses given in table 2 show that appreciable

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[2] The authors wish to thank Dr. Louis Lykken, Shell Chemical Company, and Dr. Paul Porter, Shell Development Company, for chemical analyses of all residue samples. Acknowledgement is also made of field assistance given by Mr. Francis Bliss, Herdsman in the Department of Animal Industry, Louisiana State University.

endrin residues were still present in lamb fat 42 days after the lambs had been removed from the treated pasture. There was apparently no measurable loss of endrin from fat 14 days after lambs were transferred from the treated to the untreated pasture, although there is an indication of some loss from fat after 42 days away from the treated pasture. Residues were always larger in samples of internal fat than in external fat.

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Greenbug Control with Di-Syston Used as a Soil Treatment[1]

NORRIS E. DANIELS, *Texas Agricultural Experiment Station, Bushland*

Entomologists have been investigating the feasibility of applying systemic insecticides at planting time to protect small grain plants from attack by the greenbug, *Toxoptera graminum* (Rond.). To date a systemic insecticide that will remain effective from planting time in the fall until the following spring, when greenbug damage is likely to occur, has not been found. A few workers have reported successful greenbug control with systemics applied as seed treatments to spring-planted small grains. Dahms & Wood (1957) used demeton as a seed treatment for controlling greenbugs on spring-planted oats and barley. Wood (1956) used both demeton and Thimet (phorate) as seed treatments for protecting small grains from greenbug attack. Daniels (1960) demonstrated, with greenhouse experiments, satisfactory greenbug control with Di-Syston® (*O,O*-diethyl S-2(ethylthio) ethyl phosphorodithioate) used as a soil treatment.

Field experiments were conducted at the Southwestern Great Plains Field Station at Bushland, Texas to determine the feasibility of Di-Syston as a soil treatment for greenbug control on spring-planted barley.

METHODS.—In 1958 Di-Syston, 2.5% granulated material, was applied to the soil at rates of 1 or 2 pounds actual per acre along with barley seed with a belt type planter (Porter 1959). In 1959 and 1960, rates of 0.2 and 0.4 pound per acre were applied. The barley was planted at the rate of 45 pounds per acre in single-row irrigated plots 10 feet in length. Seeds and insecticide were deposited at the depth of approximately 2 inches below the soil surface. Treatments were replicated four times. Cordova barley was planted in 1958 and Wintex was used in 1959 and 1960; both varieties are highly susceptible to greenbug attack. Planting dates for 1958, 1959 and 1960 were April 8, March 24 and March 11, respectively.

Cylindrical clear plastic cages 8 inches in diameter and 16 inches in height, with muslin tops, were placed at random in each row-plot and secured to the ground with stakes and string. During the 1958 and 1960 seasons, greenbug infestations were made by scattering approximately 75 greenbugs among the plants inside each plastic cage. Plants in cages with few or no greenbugs were reinfested after sampling. Greenbugs used for infesting cages were reared in an insectary having controlled environment. The first infestation of the 1958 test was delayed until May 15 by lack of greenbug stock. During 1959 infestation was

[1] Technical article No. 3665 of the Texas Agricultural Experiment Station. Accepted for publication February 17, 1961.

Table 1.—Effect of soil treatments with Di-Syston on populations of the greenbug and on stand counts and yields of barley at Bushland, Texas, 1958–60.

Actual Di-Syston (lbs./a.)	Date of First Infestation	Per Cent Reduction and Populations[a]						Plants/Ft. of Row	Yield (bu./a.)
		Dates of counts[b] (1958)							
	5/15*	5/19*	5/29*	6/5*	6/9*	6/13	Avg.		
2		100	100	100	100	—	100		
		0	0	0	0		0	9	—
1		100	100	100	100	—	100		
		0	0	0	0		0	11	—
Untreated		12	119	64	17	0	42	11	—
L.S.D. at 1% level								n.s.	
		Dates of counts[c] (1959)							
	4/13*	4/15	4/24	4/29	5/18	6/3	Avg.		
0.4		64	90	88	60	44	79		
		4	2	3	2	5	3	—	—
.2		55	85	84	60	11	71		
		5	3	4	2	8	4	—	—
Untreated		11	20	25	5	9	14	—	—
L.S.D. at 1% level							3		
		Dates of counts[b] (1960)							
	3/31*	4/5	4/12*	5/6	5/19	6/16	Avg.		
0.4		95	100	57	88	100	84		
		2	0	19	10	0	6	—	62
.2		90	75	32	48	92	57		
		4	1	30	43	1	16	—	52
Untreated		39	4	44	83	13	37	—	57
L.S.D. at 1% level							17		n.s.

[a] Dates of infestation shown by asterisks.
[b] 1958 and 1960 greenbugs per cage.
[c] 1959 greenbugs per foot of drill row.

made only on April 13. Greenbugs were field-collected in Hansford County, brought to the Station and scattered in each plot at the rate of approximately 100 per foot of drill row. Infestations were determined by counting the greenbugs in samples consisting of 1 linear foot of row at three locations within each plot. The per cent reduction was calculated by Abbott's formula.

Stand counts were made during 1958 by counting the plants in three 1-foot samples in each plot. Yields were determined in 1960 by harvesting 4 linear feet in each plot.

RESULTS.—Results of the three years' work are presented in table 1. From the time of the first infestation (May 15) in 1958 until June 9 both dosages of Di-Syston gave 100% protection from the aphids. There was total greenbug mortality by June 13 because warm weather caused extremely high temperatures within the cages. Germination was not significantly lowered by the chemical, although stand counts where 2 pounds per acre were applied were two plants per foot of row less than where 1 pound per acre was applied or in the untreated check.

During 1959, greenbug populations were significantly reduced by both rates of Di-Syston. Treatments with 0.4- and 0.2-pound per acre gave 79% and 71% reduction, respectively. April 15, two days after infestation, most of the greenbugs had died, probably from the shock of moving, leaving approximately 15% of the original stock. On June 3 populations of *Hippodamia convergens* (Guer.) were abundant within the plots and greenbugs were consumed by June 10.

The first infestation of 1960 was made March 31 and greenbugs remained in the untreated plots until the middle of June. Populations were significantly lowered by the treatments with Di-Syston; however, the difference between the 0.2- and 0.4-pound rates was not significant. The chemical, at the low dosages used, had no effect on the yields.

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A Possible Explanation of the Chemical Control of Simuliid Larvae[1]

GORDON FIELD,[2] *Major, U. S. Army*
Medical Service Corps

Fairchild & Barreda (1945) reported excellent control of simuliid larvae in Guatemala with stream concentrations of DDT as low as 0.1 p. p. m. Lea & Dalmat (1954) found that the effective field control concentrations did not necessarily kill black fly larvae in the laboratory, some larvae of one Guatemalan species surviving an exposure of 10 p.p.m. of DDT for 30 minutes. The discrepancy between practical field control and the killing laboratory dose was commented upon by Lindquist & Knipling (1957). The latter suggest that insecticides cause the larvae to detach and then fishes and other predators may kill them. Muirhead-Thomson (1957) reports on the effect of insecticides on simuliid larvae, and his data indicate that they may be killed by extremely minute amounts of insecticides in the laboratory. The published data suggest to this author that practical control dosages may not kill directly black fly larvae in treated streams, but perhaps affect them in such a way as to produce the same net result.

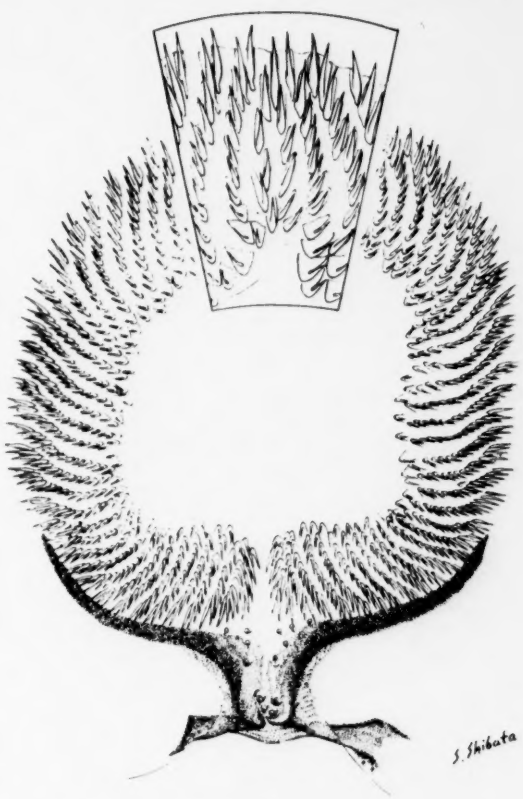

FIG. 1.—Typical anal ring of simuliid larvae showing the radiating rows of recurved hooklets.

A study of the living as well as preserved and dissected[3] simuliid larvae focused attention on the structure and probable functional operation of the anal ring. This peculiar adaptive morphologic feature of the Simuliidae possesses fundamental characteristics that may prove to be the Achilles' heel of the larvae to insecticides.

The rheophilous nature of black fly larvae is well known. The anal ring is an important structure permitting these larvae to cling to rocks, debris, and trailing vegetation in torrential streams (fig. 1). It is also well known that the hooklets of the anal ring are imbedded in a patch of silk-like material applied to the substrate by the larval mouthparts and which is produced by paired cervical glands. However, the mechanics of the anal ring are not entirely clear. The physical structure, orientation and arrangement of the hooklets suggest to this author the probable manner of their purposeful manipulation and the musculature involved, which, if correct, permit speculation as to the probable effect of insecticides on the larvae. (The muscles of the terminal segments of simuliid larvae as shown by Puri (1925a)

[1] Based upon studies of Simuliidae of Panama conducted under the auspices of the Medical Section, HQ, USARCARIB.

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[2] Present address: Medical General Laboratory (406), U. S. Army Medical Command, Japan, APO 343, San Francisco, California.

[3] Acknowledgement is made of the invaluable contribution of Wilbur Lowe, Entomological Laboratory Technician, Malaria Control Branch, Medical Section, HQ, USARCARIB, whose superbly executed dissections and slide preparations facilitated this study and others to be reported.

are very similar to those seen by this author in simuliid larvae of Panama.)

The anal ring is terminal (subanal) and is comprised of radial rows of recurved, outwardly directed hooklets. The numerous hooklets may be imbedded in the silk-like pad either by moving the hooklets outwardly upon application of the hind end to the silk patch or by first moving the hooklets inwardly and then outwardly against the silken cement. The first method is not very probable because the terminal location of the anal ring precludes very much outward movement of the hooklets as any contraction of muscles attached to the integument outside the anal ring would produce a lifting movement rather than a lateral or outward one. Also, this method would require the contraction of muscles throughout the time that the larva remained attached in one place. The second method is most probable and could be accomplished by first rolling or moving the anal ring inwardly by contraction of muscles attached to the integument within the ring. The resilient body wall (exoskeleton), returning to its former shape as the muscles relax, produces the necessary outward movement of the hooklets to snag them in the silk pad. In this manner the attachment of the simuliid larva to the silk patch on the substrate is passive with little or no expenditure of energy as muscles are not involved in maintaining the hooklets imbedded in the silk. Thus, the attached larvae of black flies can withstand the buffeting of water in rapidly flowing streams without undue fatigue.

Several authors have described the purposeful, geometrid-like progression of simuliid larvae over submerged surfaces (Puri 1925a, 1925b). Simuliid larval location is possible only because the larva has control over engaging and disengaging the hooklets of the anal ring. Under normal conditions, contractions of the muscles within the anal ring probably occur only within the pattern of sequential nerve impulses for the coordinated movements of the body and silk secretions involved in larval locomotion. However, when an insecticide such as DDT is introduced to the aqueous environment, uncoordinated muscular contractions are induced perhaps in the manner proposed by Roeder & Weiant (1946) for the action of DDT on the American cockroach. Since the attachment is passive and the disengagement of the hooklets is muscular, these uncoordinated muscular contractions progressively dislodge the hooklets until the torrent sweeps the now helpless larva downstream to be deposited perhaps in an unsuitable environmental niche such as a pool, lake, or the sea. The larva is incapable of reattaching itself to the substrate by means of the anal ring as long as the DDT- or other insecticide-induced muscular contractions continue. It is possible that fishes devour the detached larvae, although predators would not appear to be necessary to the elimination of simuliid larvae from DDT-treated streams.

Preliminary laboratory studies on the effect of insecticides on simuliid larvae have thus far confirmed the speculations given above. However, since the author is committed to other work for some time into the future, this note is published in the hope that it may stimulate timely study.

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Effect of Mite Control on Corn Yield[1]

E. C. KLOSTERMEYER, *Washington State University, Irrigation Experiment Station, Prosser*

The two-spotted spider mite, *Tetranychus telarius* (L.), is abundant on sweet corn and dent corn grown under irrigation in central Washington. Although the mites have not been observed to kill the plants, the leaves become heavily webbed and the feeding injury is obvious. Since such injury generally does not occur until the middle of August, it is questionable whether reduction in yield results. Experiments to determine this point were conducted in 1958 and 1959 at the Irrigation Experiment Station, Prosser, Washington.

METHODS.—The primary purpose of the experiment was to determine the effect of mites on yield. For this reason, treatments were chosen which would provide a range of mite populations rather than to determine the best possible acaricide. In addition to an untreated check, DDT was used to increase the mite populations above the level that could be expected in the check. Two rates (indicated in table 1) each of 1,1-bis(*p*-chlorophenyl) 2,2,2-trichloroethanol (Kelthane®), S-(*p*-chlorophenylthiomethyl)*O,O*-diethyl phosphorodithioate (Trithion®), and ethion provided the desired range of mite populations.

The eight treatments were arranged in a randomized block design of four replications in a field of Wisconsin 642 dent corn at the Irrigation Experiment Station. A different field was used in 1959 but the same experimental design was utilized each year. Plots consisted of four, 38-inch rows 90 feet long. In 1958 a self-propelled high clearance sprayer was used and the boom was fitted with drops bearing three No. 3 Cone-jet nozzles spaced 14 inches apart on each side of the row plus a flat fan nozzle over each row. The rate of application was 30 gallons per acre. In 1959, an additional nozzle was added to the drops and the rate of application reduced to 22 gallons per acre. The corn was 6 to 8 feet high and beginning to silk at the time of application on July 18, 1958 and on July 31, 1959.

Mite samples were taken in the field at weekly intervals, using a device that crushed the mites on the underside of a portion of a leaf onto a strip of adding machine paper. Leaf areas of $2 \times 2\frac{1}{4}$ inches were sampled and 20 such samples taken per plot. Only the large, easily identifiable, crushed bodies of the adult mites were counted. At harvest time, 30 feet of two rows in each plot were harvested, the corn shelled and weighed, and the moisture content determined. The yield in bushels per acre was computed at 15.5% moisture.

RESULTS AND DISCUSSION.—The population development of the mites followed a similar pattern each year, increasing gradually until the middle of September, then dropping. As anticipated, DDT resulted in increased populations of mites each year. The ethion and lower rates of Kelthane and Trithion provided intermediate populations and the higher rates of Trithion and Kelthane provided relatively low populations. Mite populations in the high Trithion treatment remained at about one-fourth that of the checks for 3 weeks and in the high Kelthane treatment for 4 weeks after application.

The yields and seasonal total mite populations for 1958 and 1959 are given in table 1. Although differences among treatments

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Table 1.—Mite populations and yield data on field corn, Prosser, Wash.

TREATMENT (LB./A.)	MITES $\sqrt{n}$	YIELD[a] (BU./A.)	DRY CORN PER EAR (LB.)	MOISTURE AT HARVEST (%)
		1958[b]		
Untreated	24.2	67.5	0.279	12.3
DDT, 2	29.0	92.7	0.320	13.3
Ethion, 0.5	20.5	77.3	0.327	12.9
1.0	22.4	74.3	0.327	12.4
Trithion, 0.25	22.0	69.0	0.275	12.3
1.0	17.4	66.2	0.274	12.0
Kelthane, 0.5	17.1	76.7	0.312	13.0
1.0	11.5	73.4	0.263	12.5
S.E. of mean	0.91	n.s.	0.00005	n.s.
		1959[c]		
Untreated	28.5	119.2	0.333	31.5
DDT, 2	31.6	117.5	0.308	31.1
Ethion, 0.5	24.4	121.6	0.337	32.2
1.0	20.3	107.5	0.325	31.7
Trithion, 0.25	22.3	111.2	0.318	30.8
1.0	14.5	107.8	0.306	30.2
Kelthane, 0.5	21.1	117.6	0.348	33.0
1.0	14.1	126.6	0.329	31.6
S.E. of mean	1.00	n.s.	n.s.	n.s.

[a] Computed at 15.5% moisture content.
[b] Square root of total number of mites in 5 weekly samples (450 sq. in. leaf surface).
[c] Square root of total number of mites in 6 weekly samples (540 sq. in. leaf surface).

were significant for the mite populations, there were no significant differences among treatment yields either in 1958 or in 1959. The 1958 summer weather was unusually hot and it was difficult to maintain adequate soil moisture in the shallow soil where the corn was grown. Some plots were damaged by lack of water. Since this may have contributed to the nonsignificance of the 1958 yield data, the experiment was repeated in 1959 but the results were about the same. In 1958, yields in treated plots were generally higher than in the check but in 1959, yields in treated plots were generally lower than in the checks. The regression equation for yield on mite populations was calculated. The regression coefficient was not significant. This indicates that the mites did not affect the yield. The high yield of corn in the DDT treatment in 1958 may have come from corn earworm (*Heliothis Zea* (Boddie)) control since the application was made at silking time. The 1959 application was made when only a few silks were showing and earworm control was less likely. Earworm damage was not determined either year.

Certain features of the data suggest that some factor related to the acaricide application may have affected yields. Except for the higher rate of Kelthane in 1959, in each year the higher rate of each acaricide resulted in a lower yield of corn than the lower rate. This appears to be due to smaller ears that accompanied the higher acaricide rates (table 1, 1958). The higher rate of Kelthane in 1959 resulted in smaller ears but since these plots had more stalks and ears, the total plot yield was higher.

From the nature of the feeding injury it might be supposed that mite feeding would hasten maturity of the crop. However, the higher rates of acaricide treatment (which also resulted in the lower mite population) had the lower moisture content at harvest (table 1). While these differences are small they are consistent from year to year and suggest that the acaricide in some way is adversely affecting the plant. No obvious effects on the plant were noted during these investigations but Verma (1956) found that demeton sprayed on corn in Hawaii interfered with silk extension and resulted in barren ears. Perhaps a similar though more subtle effect was involved in these tests.

However, there is no indication that control of mites at the population levels existing in these tests will result in significantly increased yields of grain corn.

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A Technique for Individual Rearing of Small Phytophagous Insects[1]

MAURO E. MARTIGNONI and JAMES E. MILSTEAD,
Department of Biological Control, University of California, Berkeley

In order to avoid disease transmission from host to host, individual rearing of insects is a necessity in insect pathology, as well as in some other branches of entomology, when isolation is required for the study of some characteristic of the organism. Individual rearing techniques have been recommended by many entomologists. A particularly simple method is the use of paper cartons (half-pint size or larger, which can be sterilized repeatedly or can be disposed of each time) covered with the bottom-half of a petri dish (Steinhaus & Jerrel 1954, Tanada 1953). The food may be kept fresh for considerable periods of time by immersing the stems of sprigs or the petioles of leaves in water-filled shell vials, stoppered with absorbent cotton ("bouquets"; Steinhaus 1953). The use of disposable plastic vials ($\frac{5}{8}$×2 inches; Lermer Plastics Inc., Garwood, N. J.), as recently introduced in our laboratory, eliminates the costly process of decontamination, washing, and sterilization, which is required for the more expensive glass vials.

The carton-bouquet technique is not practical, however, for rearing single first-instar larvae of lepidoptera, as they may easily escape from the carton (the closure not being perfect), or once they have abandoned the food may not find it again (because of the size of the rearing container). Furthermore, it is often quite tedious to try to locate a small larva on a bouquet when it is time to make observations or to change the food plant. During a study involving first-instar larvae of the alfalfa caterpillar, *Colias eurytheme* Boisduval, we developed a method of rearing which proved itself both simple and reliable. We give a brief description of this technique here, hoping that it may be of use for the rearing and study of other small phytophagous organisms.

Materials:

freshly collected alfalfa leaflets, with their petioles still attached;

1.5% purified agar (such as "Bacto-Agar") in distilled water; ATTENTION: do *not* use nutrient agar mixtures;

2-dram screw-cap glass vial, with the head of the cap removed by grinding (see fig. 1);

organdy.

Procedure:

1. Melt the agar (if desired, autoclaving may precede this

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FIG. 1.—View of the rearing vial, with alfalfa leaflet partly embedded in agar.

step) by immersion of the container in boiling water; let cool to a temperature around 50° C.

2. Pour 2 ml. of agar in each of 4 to 6 vials.
3. Place the leaflet in the vial, immersing it to one third of its length in the melted agar.
4. Place the glass vials in a vertical position in a pan containing cold water (about 1 inch deep). The agar will solidify almost immediately.
5. Repeat the preceding steps for a second batch of 4 to 6 vials, until the desired number of rearing units is obtained.

Rearing units thus prepared may be used immediately or may be preserved in a refrigerator at the temperature of 4° C. for 1 week or longer. However, after a considerable period of refrigeration the leaflets do not remain fresh for long once they have been returned to room temperature. Eggs are placed singly in the containers. In the case of *C. eurytheme*, which lays its eggs singly, these are transferred by excision of the leaf tissue supporting them, using a sterile number 1 cork borer (diameter 4 mm.). The vials are then closed with organdy and the modified cap is screwed in place (see fig. 1).

Leaflets of alfalfa so prepared remained fresh for a period of 3 to 4 days. Transfer of the small larvae to a fresh unit is accomplished by means of sterile wooden toothpicks, which are discarded after each transfer.

With this method the larvae can be observed at all times, without being removed from the container. If observation with the high-power objectives of a stereoscopic microscope is desired, a flat vial as described by Schneider-Orelli *et al.* (1926, 1938) should be used, instead of the 2-dram screw-cap vial.

The technique described provides all the advantages of other known methods (as, *e.g.*, the one by Langenbuch 1954), and in addition it is much simpler than other procedures, less time consuming, and more suitable for tests involving large series of animals.

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Temporary Storage of Living Soil and Bark Inhabiting Coleopterous Larvae in Water[1]

IMANTS MILLERS[2]

During investigations on the biology of the pine root collar weevil, *Hylobius radicis* Buchanan, difficulties were encountered in the field when several larvae were collected in a single container. The three major problems of dessication, overheating and cannibalism were solved by simply holding the larvae in water.

Larvae were observed in the field to be sluggish after soaking rains. This led to submerging middle instar larvae in distilled water and holding them at room temperatures. Despite the moving mouthparts during the first hour of submersion, no cannibalism occurred. Body movements were observed for two days, after which muscular contractions on the dorsum were the only signs of life. These ceased on the seventh day and death may be presumed. Since this experiment, larvae have been collected in water and held at room temperatures for two to three days without apparent deleterious effects on subsequent development. At near freezing temperatures, larvae have survived six weeks of submersion. Collins et al. (1936) reported that *Scolytus multistriatus* (Marsham) larvae survived two months in tap water and completed development. In the Wisconsin laboratory some elaterid and scarabeid larvae also have recovered after three week submersion in cool water. Adult pine root collar weevil mortality may be high after two days in water, although one

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[2] Research Assistant in Entomology, University of Wisconsin, Madison.

adult recovered after three-day submersion. Pupal mortality was high even when they were submerged for less than a day.

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A Method for Selection for DDT-Susceptibility[1]

ALEXANDER S. TAHORI,[2] *University of Illinois, Urbana*

Some insecticide-susceptible house fly strains lose part of their susceptibility during routine rearing in the laboratory. This may be due to accidental exposure to insecticides, contamination with resistant individuals, or to the use of rearing procedures which might be more favorable to resistant flies. The purpose of this study was to investigate a method for selecting house flies (*Musca domestica* L.) for greater DDT-susceptibility based on the negative temperature coefficient of DDT.

Cole & Clark (1958) have attempted to develop a DDT-susceptible strain of body lice from a resistant one by exposing third-instar nymphs to 3-p-chlorophenoxy-1,2, propane oxide and using only the most susceptible individuals, as measured by knockdown, for breeding purposes. However, they reported no loss of resistance even after 20 generations of selection.

The DDT-susceptible Stauffer[3] house fly strain was used. It was reared by the official Peet-Grady Method (Anonymous 1947). Approximately 100 newly emerged flies were exposed to a DDT residue of 20 μg/100 cm.^{2} evaporated from an acetone solution. The exposure chamber consisted of a wide mouth Mason jar having an inner surface of 320 cm.^{2}. A hole 2 cm. in diameter was made at the bottom of the jar, plugged by a cork. During the 45-minute exposure period the flies were kept at 15° C. At the end of this period the cork was removed and the prostrate flies were gently shaken out of the jar and transferred to a 37° C. chamber, where they were kept for 2 hours. More than 50% of the prostrate flies recovered and were used as the parent stock for the next generation.

Insecticide susceptibility determinations were made by topical application with a microsyringe (0.1-cc. syringe of Hamilton Co., Whittier, California) of DDT-ethanol solutions to the ventral thorax of 2- to 4-day-old female flies. Fifty flies were tested for each of the following concentrations: 0.04, 0.06, 0.08, 0.12, and 0.16 μg. of DDT per fly. After treatment the flies were kept at 20° C. and 60% relative humidity. Mortalities were counted after 24 hours.

Although the flies had been selected for knockdown, the slope of the dosage-mortality curve became increasingly steeper after selection for 3, 6, and 9 generations. However, with succeeding generations the rate of increasing susceptibility diminished (table 1). The use of the negative temperature coefficient of DDT, therefore, does not seem to provide a means of obtaining a completely susceptible fly strain, which should possess a very steep dosage mortality curve. Even after selection for nine generations a dosage of 0.16 μg. of DDT per fly still gave only 82% mortality. However, by subjecting every third or fourth generation to selection by this method, a susceptible laboratory colony can be maintained at a consistently low level of resistance even where the danger of contamination exists.

[1] Accepted for publication January 30, 1961.

[2] Andre Mayer Fellow of the Food and Agriculture Organization of the United Nations for 1959. Permanent address: Israeli Institute for Biological Research, Ness Ziona, Israel.

[3] Obtained by courtesy of Dr. J. J. Menn, Stauffer Chemical Company, Mountain View, California.

Table 1.—Representative dosages of successive generations after selection for DDT-susceptibility.

GENERATION	μG. OF DDT PER FLY			95% CONFIDENCE LIMIT FOR LD_{50}[a]
	LD_{20}	LD_{50}	LD_{80}	
0	0.044	0.120	0.372	0.099–0.146
3	0.036	0.088	0.236	0.074–0.104
6	0.035	0.080	0.196	0.069–0.094
9	0.035	0.077	0.182	0.067–0.089

[a] Calculated according to the method of Litchfield & Wilcoxon (1949). There was a significant difference between the 0 and all other generations but not between 3 and 6 or 9 generations.

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Production Uniformity in the Commercial Culture of Entomophagous Insects[1]

S. E. FLANDERS, *Department of Biological Control, University of California Citrus Experiment Station, Riverside*

Under natural conditions an animal species is necessarily in balance, balance being a prerequisite for the existence of an organism. Such balance, however, is often characterized by population oscillations of great amplitudes.

Oscillation in the mass culture of entomophagous insects, however, is inimical to efficient operation. As pointed out by the writer (Flanders 1949), uniformity is a necessary condition for economical production.

When an entomophagous insect is continuously cultured at the maximum numerical level fixed by its habits and its nutritive and physical environment, the culture is necessarily in equilibrium at a steady density. In the mass culture of organisms, maximum efficiency in production per unit of space is attained when the populations produced per day are numerically equal.

Maintenance of a steady density in non-self-perpetuating cultures requires manipulation of the parental stock (inoculum). For efficiency in such production the ratio of inoculum to the amount of food available must be such as to obtain the maximum production in the minimum of time (Flanders 1949).

In the mass culture of an entomophagous insect, the parental stock consists either of the whole of each day's production or of a fraction thereof. When the inoculum consists of a whole day's production, economy of operation requires that the average number of adult laboratory progeny per parent should not exceed one. When a fraction of a whole day's production is used, the laboratory progeny of this fraction must duplicate the total production per day.

Only in one of the known mass cultures of entomophagous insects does the maintenance of equilibrium through use of the daily production as a whole appear economically possible. This instance is the mass culture of the hymenopterous ectoparasite *Aphytis lingnanensis* Comp. on mass-produced populations of the diaspine scale *Aspidiotus hederae* (Vallot) as reported by De-

[1] Accepted for publication February 7, 1961.

Bach and White (1960). In this case the type of host plant (fruits of *Cucurbita maxima*) as well as the type of insect infesting it permits the economical recovery of the whole day's production for use as an inoculum. However, the full use of the host insect population is precluded by the necessarily short period of its exposure. When *Aphytis* is propagated in this fashion only about 50% of the host population exposed is parasitized.

CULTURE METHODS.—There are two methods of commercial mass culture: "limited-contact" and "periodic-contact" (Flanders 1954). The limited-contact method in which a natural enemy population is introduced into a host population, as in the case cited above, is usually used for the culture of predators.

Limited contact results from one of the following: (1) the complete destruction of the host population; (2) the host outgrowing its susceptible stages; (3) the short reproductive life of the inoculum; or (4) the arbitrary removal of the inoculum. Given a superabundance of hosts, equilibrium is attained by manipulating the amount of the inoculum and the length of the host-exposure period.

The periodic-contact method of mass culture—bringing a succession of briefly discrete host populations, uniform in development, into contact with a protracted parasite population which is constant in density—is generally the most economical in the use of host material. Examples of this method are the cultures of *Trichogramma* (Flanders 1929) and of *Dibrachys* (Doutt & Finney 1947). Maintenance of equilibrium in these cases requires a careful adjustment of the susceptible stages of the host population to the action of parasite population.

In most if not all mass cultures of record the multiplication per day for maintaining equilibrium has varied from one- to fortyfold (Flanders 1954). Curiously, in all such cultures the maximum increase which may be obtained per inoculum is indicated by the number of eggs the average female is capable of depositing per day.

In stating this idea in an earlier publication (Flanders 1954), the author inadvertently used "minimum" in qualifying the amount of increase.

CONTROL OF PEST ORGANISMS AFFECTING EQUILIBRIUM.—Contamination of a host culture by other organisms that utilize the same kind of food may interfere with the maintenance of the equilibrium position. Such interference is reduced to a minimum by the introduction into the culture of the natural enemies of the competing organisms. The predatory thrips *Scolothrips sexmaculatus* Perg. was used to control the phytophagous mite, *Tetranychus pacificus* McG., in cultures of diaspine scales on squash fruits (Flanders 1943); the encyrtid *Acerophagus pallidus* Timb. to control the mealybug *Phenacoccus solani* Ferris in cultures of black scale, *Saissetia oleae* (Bern.), on potato sprouts (Flanders 1944); and the braconid, *Bracon hebetor* Say, to control the Indian meal moth, *Plodia interpunctella* (Hbn.), in cultures of the Angoumois grain moth, *Sitotroga cerealella* (Oliv.), in trays of maize (Flanders 1930). The control of the grain beetle, *Laemophloeus* sp., in cultures of *S. cerealella* was accomplished by the bethylid, *Cephalonomia waterstoni* Gahan (Schread & Garman 1933). The natural enemies reduced interspecific competition and thus increased intraspecific competition.

These instances of competitive organisms controlled by the natural enemies constitute a third type of culture method designated the "continuous-contact" method (Flanders 1954). It is the only host-parasite culture method in which equilibrium is automatic, an equilibrium resulting from the self-regulation of the population.

Cultures of prey-predator (host-parasite) populations demonstrate that in reproducing populations under constant conditions, balance is essential for operational efficiency. Balance at constant densities is attained by either of two methods. One, designated "limited-contact," involves briefly discrete populations of both the host (prey) and its natural enemy; the other, "periodic-contact," involves a protracted natural enemy population and a series of briefly discrete host (prey) populations. Biological control of pest populations may be necessary to maintain balance at maximum densities.

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MISCELLANEOUS PUBLICATIONS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA

Volume 2, Number 3, pp. 213–37 was issued in late May 1961 and contains "The Larval Ixodid Ticks of the Eastern United States (Acarina-Ixodidae)," by Carleton M. Clifford, George Anastos and Alena Elbl. Single copy, $2.50.

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OBITUARIES

Wendell Folsom Sellers
1903-1960

Wendell F. Sellers was born August 15, 1903, in Everett, Massachusetts. He lived much of his youth in Melrose, where his mother, Mrs. Annie Sellers, still resides. From 1920 to 1924, he attended the Massachusetts Agricultural College (now University of Massachusetts), graduating with the degree of B.S. in entomology. He was married December 28, 1927, to Amy Lindsay of Melrose, Mass. On October 18, 1960, Wendell died in Bangkok, Thailand, following a coronary occlusion. He is survived by his wife Amy; two daughters, Mrs. C. Richard Sanders of Ontario, Calif., and Mrs. Roy Eastwood of Santa Clara, Calif.; seven grandchildren; his mother; and a sister, Mrs. Elston Dunn of Melrose, Mass.

Upon graduation from college Wendell worked for the then Division of Forest Insect Investigations, U. S. Bureau of Entomology, at the Melrose field station. Here he began the study of native and foreign tachinid parasites in which he continued to be interested for some years. In 1930 he was sent abroad to explore for natural enemies of insect pests introduced into the United States. Until 1935 Wendell was located in Budapest, Hungary; from 1935 to 1938 in France. He travelled extensively in Europe, contacting officials of various governments, making surveys, and personally supervising large groups of temporary employees, sometimes at several rearing points in the same season. Wendell had a marked interest in the European political situation but worsening conditions in that respect led him late in 1938 to return to the United States with his family. Until 1941 he directed the propagation and establishment of introduced natural enemies of forest insect pests, with headquarters at New Haven, Conn.

In January, 1942, Wendell left the service of the United States to become Assistant Director, under Dr. W. R. Thompson, of the Commonwealth Institute of Biological Control in Ottawa, Canada, a position he held until March, 1959. He established laboratory facilities for this work in Fontana, Calif., close to the Citrus Experiment Station at Riverside with its prominent work in the biological control of insects. Studies on behalf of the British Commonwealth of Nations required much travel. A major accomplishment was the establishment of biological control centers in Rawalpindi, Pakistan, and Bangalore, India, for the Colombo Plan Administration of Canada, the Government of Pakistan, and the Government of India. This project kept Wendell in Pakistan and India for three years.

Upon the retirement of Dr. Thompson, Wendell resigned his post in March, 1959, to become entomology adviser with the U. S. Operations Mission (ICA) to Thailand. Here he was particularly popular, the Thai Ministry of Agriculture having asked that he be returned to Bangkok for another two-year tour of duty. Of particular significance was Wendell's assistance to the Thai Government in developing an economic poisons act to regulate the use of pesticides in that country.

The publications of Wendell Sellers include articles on the distribution of the European spruce sawfly in Scandinavian and eastern Baltic countries, the mass production of predators of armored scales, various phases of biological control, as well as a monograph on "The Nearctic Species of Parasitic Flies Belonging to *Zenillia* and Allied Genera" (Proc. U. S. Nat. Mus. 93: 1–108, 1943).

Wendell was a member of the Entomological Society of America, the Entomological Society of Canada, the Finnish Entomological Society, the Siam Society, the Royal Bangkok Sports Club, and Alpha Gamma Rho.

HAROLD H. SHEPARD

Bentley Ball Fulton
1889-1960

Dr. Bentley Ball Fulton, Professor Emeritus in Entomology at North Carolina State College, died at his home December 8 at Raleigh, North Carolina, after a lingering illness.

Dr. Fulton was born August 29, 1889, at Newark, Ohio. He received his B.A. degree from Ohio State University, 1912; his M.S. from the University of Chicago, 1916; and his Ph.D. from Iowa State College, 1926. He was a member of the Entomological Society of America, American Association for the Advancement of Science, the Ecological Society of America, Society for the

Study of Evolution, North Carolina Academy of Science, and Sigma Xi.

Dr. Fulton was Assistant Entomologist, New York Agricultural Experiment Station, 1912–1919; Associate Entomologist, Oregon Agricultural Experiment Station, 1919–1924; Assistant Entomologist, Iowa State College and Experiment Station, 1924–1928; Professor of Entomology, North Carolina State College since 1928.

Dr. Fulton won world recognition as an artist of entomological subjects. Many of his drawings have been used time and again in textbooks, as well as various experiment station publications in many states.

Dr. Fulton was a keen observer and a true biologist. He pioneered in the work of distinguishing species of crickets by their songs, as well as by morphological characters. He is one of the truly great biologists; however, due to his quiet, unassuming attitude, he was appreciated most by those who had the pleasure of close association with him. He was always most patient and sincere in everything he undertook, attributes of a great biologist.

He is survived by his wife, Mrs. Ida Kemp Fulton, and two daughters, Mrs. Margaret Fulton Hart of Atlanta, Georgia, and Mrs. Dorothy Fulton Kimbrell of Long Island, New York.

CLYDE F. SMITH

BOOK REVIEW

TOXIC PHOSPHORUS ESTERS—CHEMISTRY, METABOLISM AND BIOLOGICAL EFFECTS, by Richard D. O'Brien Academic Press, Inc., New York, 434 pp. 1960. Price $14.50.

Professor O'Brien has compiled the voluminous literature on toxic phosphorus esters into an admirably concise, readable and provocative book. The scope of material covered and method of presentation make this the best reference book available for entomologists dealing with the chemistry and toxicology of this important class of insecticides. Biologists and chemists in general will benefit from the interdisciplinary approach used in considering the chemistry, metabolism and biological effects of phosphorus esters. This is a book on cholinesterase and cholinesterase inhibitors and the significance of each in the survival of animals. An introduction clarifies nomenclature and other necessary points of general background. Non-enzymic reactions of organophosphates are presented in detail. Cholinesterase is considered from the standpoint of structure, *in vitro* reaction with organophosphates and significance in the economy of the organism. Enzymic activation and degradation of the compounds are considered, as is the metabolic fate of the phosphorus insecticides in insects, mammals and plants. Selective toxicity, a subject of special personal research emphasis for Dr. O'Brien, is well presented. A final section on techniques and electronic interpretations provides the theory and practice used in investigations on the chemistry of these toxicants. No detailed attempt is made to consider synthetic methods for the wide variety of phosphorus esters, nor to compile listings of pesticidally active phosphates from the extensive patent literature. The book is not without errors in the text and structural formulae, but these rare cases will usually be evident to the reader as they appear as obvious inconsistencies in the presentation.

Dr. O'Brien has made a significant contribution to the field of mode of action of organophosphates by his critical analysis of the available information and enlightening suggestions on where the future research in this field might need emphasis.

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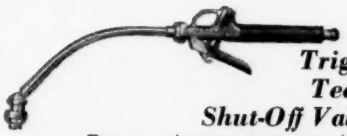

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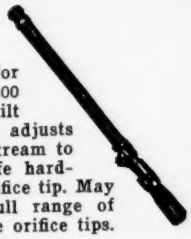

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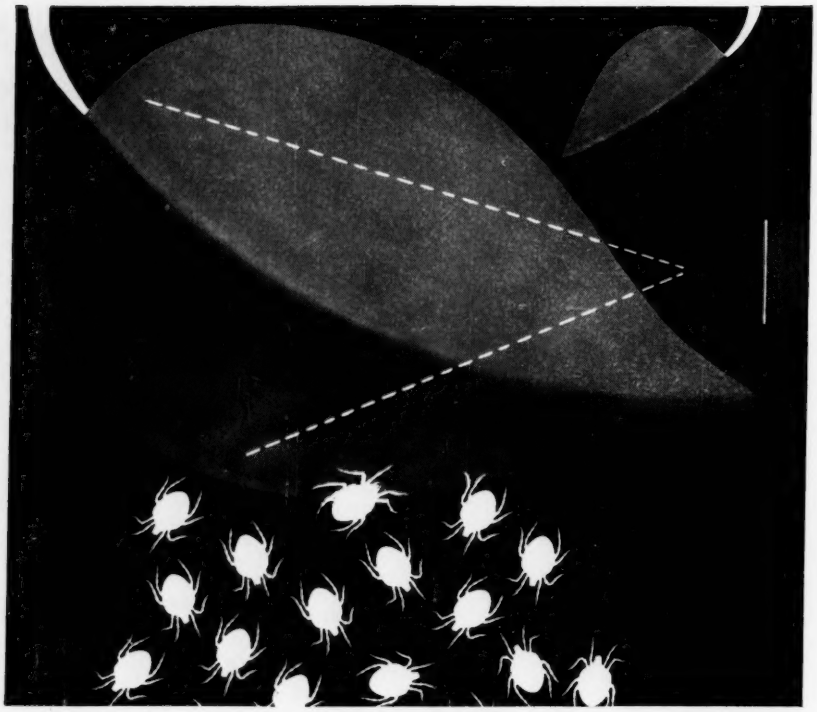

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